Infrapatellar Fat Pad and Knee Osteoarthritis

by

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

(Medical Research)

University of Tasmania

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Certificate of Originality

I hereby declare that this thesis is my own work and contains no material which has been accepted for a degree or diploma by the university or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of knowledge and belief, it contains no material previously published or written by another person, except where due acknowledgement is made in the text, nor does the thesis contain any material that infringes copyright.

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Statement of Co-Authorship

This thesis includes papers for which Weiyu Han (WH) was not the sole author. WH was the lead in the research of each manuscript; however, he was assisted by the co-authors, whose contributions are detailed below.

Chapter 4:

Han W, Aitken D, Zhu Z, Halliday A, Wang X, Antony B, Cicuttini F, Jones G, Ding C. Signal intensity alteration in the infrapatellar fat pad at baseline for the prediction of knee symptoms and structure in older adults: a cohort study. Annals of the Rheumatic Disease. 2016; 75(10): 1783-8.

The contribution of each author:

WH participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

DA, ZZ and BA participated in the acquisition of data, manuscript preparation, and statistical analysis.

AH participated in the acquisition, analysis and interpretation of data, and manuscript preparation.

XW participated in the acquisition of data, manuscript preparation, and statistical analysis.

FC participated in the study design, analysis and interpretation of data, and statistical analysis.

GJ participated in the study design, acquisition, analysis and interpretation of data, and manuscript preparation.

CD carried out the study design, participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

Chapter 5:

Han W, Aitken D, Zhu Z, Halliday A, Wang X, Antony B, Cicuttini F, Jones G, Ding C. Hypointense signals in the infrapatellar fat pad assessed by magnetic resonance imaging are associated with knee symptoms and structure in older adults: a cohort study. Arthritis Research & Therapy. 2016; 18(1): 234.

The contribution of each author:

WH participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

DA participated in the analysis and interpretation of data, manuscript preparation, and statistical analysis.

ZZ and XW participated in the acquisition of data, manuscript preparation, and statistical analysis.

AH and BA participated in the acquisition, analysis and interpretation of data, and manuscript preparation.

FC participated in the study design, acquisition of data, manuscript preparation, and statistical analysis.

GJ participated in the study design, acquisition, analysis and interpretation of data, and manuscript preparation.

CD carried out the study design, participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

Chapter 6:

Han W, Aitken D, Zheng S, Wluka A, Zhu Z, Blizzard L, Winzenberg T, Cicuttini F, Jones G, Ding C. Association between quantitatively measured infrapatellar fat pad high signal intensity alteration and knee structural abnormalities in patients with symptomatic knee osteoarthritis. Manuscript is under revision in Arthritis Care & Research.

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WH participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

DA, LB and SZ participated in the analysis and interpretation of data, manuscript preparation, and statistical analysis.

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TW and FC participated in the study design, acquisition of data, manuscript preparation, and statistical analysis.

GJ participated in the study design, acquisition, analysis and interpretation of data, and manuscript preparation.

CD carried out the study design, participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

Chapter 7:

Han W, Aitken D, Zheng S, Wang B, Wluka A, Zhu Z, Blizzard L, Wang X, Cicuttini F, Jones G, Ding C. Serum levels of resistin are associated with knee synovitis and structural changes in patients with symptomatic knee osteoarthritis. Manuscript is under revision in Osteoarthritis & Cartilage.

The contribution of each author:

WH participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

DA, AW and SZ participated in the analysis and interpretation of data, manuscript preparation, and statistical analysis.

ZZ, BW and XW participated in the acquisition, analysis and interpretation of data, and manuscript preparation.

LB and FC participated in the study design, acquisition of data, manuscript preparation, and statistical analysis.

GJ participated in the study design, acquisition, analysis and interpretation of data, and manuscript preparation.

CD carried out the study design, participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

Chapter 8:

Han W, Aitken D, Zheng S, Wang B, Wluka A, Zhu Z, Blizzard L, Wang X, Cicuttini F, Jones G, Ding C. Natural History of Infrapatellar Fat Pad High Signal Intensity Alteration and Factors Affecting Its Changes. Manuscript is under review in Osteoarthritis & Cartilage.

The contribution of each author:

WH was responsible for data collection, data management and cleaning, carried out analysis and interpretation of data and manuscript preparation.

DA, SZ and ZZ participated in the acquisition, analysis and interpretation of data, and critically revised the manuscript.

BW, AW and XW participated in the acquisition of data, and critically revised the manuscript.

LB and FC designed and carried out the study planning, participated in analysis and interpretation of data, and critically revised the manuscript.

GJ participated in the study design, acquisition, analysis and interpretation of data, and manuscript preparation.

CD carried out the study design, participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis.

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Weiyu Han (Candidate)

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Changhai Ding (Primary supervisor)

Statement of Ethical Conduct

The research of this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University of Tasmania.

Abstract

Osteoarthritis (OA) is the most common and prevalent chronic joint disorder in older adults, characterized by progressive deterioration of joint structures. It affects not only the articular cartilage and subchondral bone but also other structures of the joint, such as menisci, synovial membrane, joint capsule, ligaments, muscles and infrapatellar fat pad (IPFP). IPFP, the biggest 'organ' in the knee and may play an important role in the incidence and progression of knee OA. This thesis aims to describe the associations of semi-quantitative and quantitative measures of IPFP signal intensity alterations assessed on magnetic resonance imaging (MRI) with knee structural and symptomatic changes in older adults or patients with symptomatic knee OA.

This thesis is based on two established studies: Tasmanian Older Adults Cohort (TASOAC) study and Vitamin D Effects on Osteoarthritis (VIDEO) study. TASOAC is an ongoing prospective, population-based study, aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA (assessed by both X-ray and MRI). Baseline measures were conducted from April 2002 to September 2004, and the first follow-up were conducted from September 2004 to February 2007 (mean 2.7 years, range 2.6-3.3 years). Subjects (n=1100) between the age of 50 and 80 years were randomly selected from the roll of electors in southern Tasmania (population, 229,000), a comprehensive population listing with an equal number of men and women. VIDEO is a multicentre, randomized, placebo-controlled double-blind clinical trial. The aim of this study is to compare the effects of vitamin D supplementation versus placebo on knee structural and symptomatic changes in patients with symptomatic knee OA over a 2-year period. Patients (n = 413) with symptomatic knee OA and low 25(OH)D levels were randomized to treatment and control groups in Hobart and Melbourne. Outcomes/predictors included knee symptoms assessed by

WOMAC index and visual analogue scale (VAS), knee structural changes assessed by radiography or MRI, and serum biomarkers (IL-1, IL-6, TNF-α).

Chapter 4 describes the associations between semi-quantitative measures of IPFP high signal intensity alteration at baseline and knee symptoms and structural changes in older adults. These semi-quantitative measures of IPFP high signal intensity alterations at baseline are associated with knee structural abnormalities and clinical symptoms cross-sectionally and longitudinally in older adults, suggesting that it may serve as an important imaging biomarker in knee OA.

Chapter 5 investigates the associations between semi-quantitative measures of IPFP hypointense signals and knee structural change and symptoms in older adults. These hypointense signals in the IPFP are associated primarily with increased knee cartilage defects and also with bone marrow lesions (BMLs) and knee symptoms in cross-sectional and longitudinal analyses, suggesting the abnormality represented by this signal has a potentially important role in OA process.

Chapter 6 describes the associations between quantitative measures of IPFP high signal intensity alteration and knee structural abnormalities in patients with symptomatic knee OA. Quantitative measures of increased signal intensity in the IPFP are associated with knee structural abnormalities longitudinally, suggesting that these measurements could be used as an additional entry criteria in order to enrich for ‘faster progressors’ in studies of knee OA.

Chapter 7 investigates the associations between serum levels of resistin, IPFP signal intensity alterations and other knee structural changes in patients with knee OA. Serum levels of resistin

are significantly and positively associated with IPFP signal intensity alterations and other structural abnormalities, suggesting a potential role of resistin in knee OA.

Chapter 8 describes the natural history of IPFP high signal intensity alteration (quantitative measures) and factors affecting its changes over 2 years. IPFP high signal intensity alterations were not static in patients with knee OA. Changes of these signal intensity alterations were age-related, independent of BMI, predicted by radiographic OA (ROA), cartilage volume and effusion-synovitis, as well as associated with changes in neighboring knee structures, suggesting therapeutic interventions aimed at these changes could be considered in clinical practice.

In conclusion, age-related signal intensity alterations in the IPFP are associated with greater ROA progression, predict symptomatic and structural changes, and are related to neighboring knee structural abnormalities. Combined with its associations with pro-inflammatory mediators, these signal intensity alterations may represent age-related local adipose tissue inflammation, which contributes to the incidence and/or progression of knee OA. Furthermore, these signal intensity alterations may be used as imaging biomarkers in clinical research or practice.

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Publications Arising from the Thesis

Chapter 4: Han W, Aitken D, Zhu Z, Halliday A, Wang X, Antony B, Cicuttini F, Jones G, Ding C. Signal intensity alteration in the infrapatellar fat pad at baseline for the prediction of knee symptoms and structure in older adults: a cohort study. Annals of the Rheumatic Disease. 2016; 75(10): 1783-8.

Chapter 5: Han W, Aitken D, Zhu Z, Halliday A, Wang X, Antony B, Cicuttini F, Jones G, Ding C. Hypointense signals in the infrapatellar fat pad assessed by magnetic resonance imaging are associated with knee symptoms and structure in older adults: a cohort study. Arthritis Research & Therapy. 2016; 18(1): 234.

Chapter 6: Han W, Aitken D, Zheng S, Wluka A, Zhu Z, Blizzard L, Winzenberg T, Cicuttini F, Jones G, Ding C. Association between quantitatively measured infrapatellar fat pad high signal intensity alteration and knee structural abnormalities in patients with symptomatic knee osteoarthritis. Manuscript is under revision in Arthritis Care & Research.

Chapter 7: Han W, Aitken D, Zheng S, Wang B, Wluka A, Zhu Z, Blizzard L, Wang X, Cicuttini F, Jones G, Ding C. Serum levels of resistin are associated with knee synovitis and structural changes in patients with symptomatic knee osteoarthritis. Manuscript is under revision in Osteoarthritis & Cartilage.

Chapter 8: Han W, Aitken D, Zheng S, Wang B, Wluka A, Zhu Z, Blizzard L, Wang X, Cicuttini F, Jones G, Ding C. Natural History of Infrapatellar Fat Pad High Signal Intensity Alteration and Factors Affecting Its Changes. Manuscript is under review in Osteoarthritis & Cartilage.

Other Publications

Han W, Fan S, Bai X, Ding C. Strontium ranelate, a promising disease modifying osteoarthritis drug. Expert Opin Investig Drugs 2017; 26(3): 375-380.

Jin X, Wang BH, Wang X, Antony B, Zhu Z, Han W, Cicuttini F, Wluka AE, Winzenberg T, Blizzard L, Jones G, Ding C. Associations between Endogenous Sex Hormones and MRI Structural Changes in Patients with Symptomatic Knee Osteoarthritis. Osteoarthritis Cartilage 2017; 25(7):1100-1106.

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Pan F, Han W, Wang X, Liu Z, Jin X, Antony B, Cicuttini F, Jones G, Ding C. A longitudinal study of the association between infrapatellar fat pad maximal area and changes in knee symptoms and structure in older adults. Ann Rheum Dis. 2015; 74(10): 1818-1824.

Wang J, Antony B, Zhu Z, Han W, Pan F, Wang X, Jin X, Liu Z, Cicuttini F, Jones G, Ding C. Association of patellar bone marrow lesions with knee pain, patellar cartilage defect and patellar cartilage volume loss in older adults: a cohort study. Osteoarthritis Cartilage. 2015; 23(8):1330-6.

Han W, Cai S, Liu Z, Jin X, Wang X, Antony B, Cao Y, Aitken D, Cicuttini F, Jones G, Ding C. Infrapatellar fat pad in the knee: is local fat good or bad for knee osteoarthritis? Arthritis Res Ther. 2014; 16(4): R145.

Scientific Presentations and Awards Arising from the Thesis

2014

Osteoarthritis Research Society International congress, Paris, France.

"Associations between Abnormal Infrapatellar FAT PAD Quality and Knee Osteoarthritic Changes in Older Adults: A Cohort Study"

(Oral presentation – presented by co-author)

Menzies Institute for Medical Research, Tasmania, Australia.

"A longitudinal study of the association between infrapatellar fat pad maximal area and changes in knee symptoms and structure in older adults"

(Ten of the Best Publication Awards 2014)

2015

Menzies Institute for Medical Research, Tasmania, Australia.

"Signal intensity alteration in the infrapatellar fat pad at baseline for the prediction of knee symptoms and structure in older adults: a cohort study"

(Ten of the Best Publication Awards 2015)

2016

Osteoarthritis Research Society International congress, Amsterdam, Netherlands.

"*Cross-sectional and longitudinal associations between signal intensity alterations in infrapatellar fat pad and knee structural changes in patients with symptomatic knee OA*"

(Poster presentation)

The American College of Rheumatology and American Rheumatology Healthy Professional (ACR/ARHP) Annual Meeting, Washington D.C., USA.

"Association Between Quantitatively Measured Infrapatellar Fat Pad High Signal Intensity Alteration and Knee Structural and Symptomatic Abnormalities in Patients with Symptomatic Knee Osteoarthritis"

(Oral presentation)

"Serum Levels of Resistin Are Associated with Synovial Inflammation and Knee Structural Changes in Patients with Symptomatic Knee Osteoarthritis"

(Poster presentation)

The 18th Asia Pacific League of Associations for Rheumatology Congress **(APLAR)** Annual Meeting, Shanghai, China.

"Cross-sectional and longitudinal associations between signal intensity alterations in infrapatellar fat pad and knee structural changes in patients with symptomatic knee OA"

(Oral presentation)

2017

The Outstanding Self-Financed Students Abroad Award of Chinese Government.

Nomenclature

25(OH)D	25-hydroxyvitamin D
2D	Two- dimensional
3D	Three-dimensional
ACL	Anterior cruciate ligament
ACR	American College of Rheumatology
ADLs	Activities of daily living
BLOKS	Boston Leeds Osteoarthritis Score
BMI	Body mass index
BMLs	Bone marrow lesions
COX-2	Cyclo-oxygenase-2
CTX-II	C-terminal propeptide of type II collagen
CV	Coefficient of variation
DMOADs	Disease-modifying osteoarthritis drugs
EULAR	European League Against Rheumatism
ICC	Intraclass correlation coefficient
IL	Interleukin
IPFP	Infrapatellar fat pad
JSN	Joint space narrowing
K/L	Kellgren-Lawrence
LSC	Least significant criterion
LTB_4	Leukotriene B_4
MCID	Minimal clinically important difference
MMP	Matrix metalloproteinase

MRI	Magnetic resonance imaging
NSAIDs	Nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
OR	Odd ratio
PGE_2	Prostaglandin E_2
PPARα	Peroxisome proliferator activated receptor α
RCT	Randomised clinical trial
ROA	Radiographic osteoarthritis
ROI	Region of interest
ROM	Range of motion
ScAT	Subcutaneous adipose tissue
sColl2-1NO2	Nitrated epitope of the α-helical region of type II collagen
sIL-6R	Soluble IL-6 receptor
sPIIINP	N-terminal propeptide of type III procollagen
TASOAC	Tasmania Older Adults Cohort
TKA	Total knee arthroplasty
TLR	Toll-like receptor
TNF	Tumour necrosis factor
VAS	Visual analogue scale (S)
VEGF	Vascular endothelial growth factor
VIDEO	Vitamin D Effects on Osteoarthritis
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
WORMS	Whole-Organ Magnetic Resonance Imaging Score

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Synopsis

Osteoarthritis (OA) is one of the most common diseases and is a leading cause of chronic disability in older adults, characterized by progressive deterioration of joint structures. It affects not only the articular cartilage and subchondral bone but also other structures of the joint, such as menisci, synovial membrane, joint capsule, ligaments, muscles and infrapatellar fat pad (IPFP), Although the aetiology and progression of this disease are not well understood, it is considered as a multifactorial condition with risk factors including genetics, aging, female sex, injury and obesity. Aging and obesity are two prominent risk factors for the onset of knee OA, and current studies suggest that age-related adipose tissue inflammation may be the underlying mechanism between age, obesity and knee OA. IPFP, the biggest intra-articular adipose tissue in the knee, is a source of cytokines, adipokines and growth factors, and has similar composition of immune cells as synovium. Despite its capabilities of reducing the impact loading and absorbing forces generated through the knee joint, it may be an important local source of age-related inflammation and a central contributor to the degradation of neighbouring tissues within the knee. The inflammation or fibrosis within IPFP may be detected as signal intensity alterations on magnetic resonance imaging (MRI). This thesis describes the associations of IPFP signal intensity alterations with symptomatic and knee structural changes, as well as pro-inflammatory mediators, aiming to examine possible relationships between pathological changes in IPFP and knee OA development or progression. Furthermore, it investigates the natural history of these signal intensity alterations and factors affecting these alterations.

Chapter 1 gives an introduction to OA, including its definition, symptoms and signs, epidemiology and economic burden, currently understanding of aetiology and risk factors, diagnostic criteria, and treatments. A brief discussion of the association between

inflammation, adipose tissue, IPFP and knee OA is given. An overview of current imaging biomarkers in clinical research and practice is provided, with a focus on IPFP signal intensity alterations.

Chapter 2 lists the research questions to be addressed in the thesis.

Chapter 3 describes the methodology included in the thesis, including study designs of two involved studies: Tasmanian Older Adults Cohort (TASOAC) study and Vitamin D Effects on Osteoarthritis (VIDEO) study, protocols for measurements, and statistical analyses.

Chapter 4 describes the associations between IPFP high signal intensity alteration at baseline and knee symptoms and structural changes in older adults. A total of 874 subjects (mean 62.1 years, 50.1% female) selected randomly from local community were studied at baseline and 770 were followed up (only 357 had MRI at follow-up) over 2.6 years. T1- or T2-weighted fat suppressed MRI was utilized to assess IPFP signal intensity alteration (0-3), cartilage volume, cartilage defects and BMLs at baseline and 2.6 years later. Knee pain was assessed by self-administered Western Ontario and McMaster Osteoarthritis Index (WOMAC) questionnaire. Radiographic OA was assessed. In cross-sectional analyses, IPFP signal intensity alteration was significantly and positively associated with total knee pain as well as knee cartilage defects, bone marrow lesions (BMLs) and knee radiographic OA and negatively associated with patellar cartilage volume after adjustment for age, sex, BMI and/or radiographic OA. Longitudinally, baseline signal intensity alteration within IPFP was significantly and positively associated with increases in knee pain when going up/down stairs as well as increases in tibiofemoral cartilage defects and BMLs, and negatively associated with change in lateral tibial

cartilage volume in multivariable analyses. In conclusion, IPFP signal intensity alteration at baseline was associated with knee structural abnormalities and clinical symptoms cross-sectionally and longitudinally in older adults, suggesting that it may serve as an important imaging biomarker in knee OA.

Chapter 5 describes the associations between hypointense signals in the IPFP and knee structural change and symptoms in older adults. Participants (n = 874) were selected randomly from local community and followed up 2.7 years later (range 2.6-3.3 years). T1- or T2-weighted fat suppressed MRI was assessed for IPFP hypointense signal, cartilage volume, cartilage defects, and BMLs. Knee pain was assessed by self-administered WOMAC questionnaire. Radiographic OA was assessed using the Osteoarthritis Research Society International (OARSI) atlas. Cross-sectionally, hypointense signals in the IPFP were significantly associated with a higher risk of knee cartilage defects at all sites, tibiofemoral BMLs and knee pain in multivariable analyses. Longitudinally, baseline signal abnormalities were significantly and positively associated with increases in knee cartilage defects (OR: 2.27 95%CI: 1.61-3.21), BMLs (OR: 1.91, 95%CI: 1.39-2.62), and knee pain (OR: 1.36, 95%CI: 1.05-1.76) in multivariable analyses. The associations with cartilage defects remained significant after adjustment for BMLs, but the associations with BMLs and knee pain decreased in magnitude or became non-significant after further adjustment for cartilage defects. In conclusion, hypointense signals in the IPFP were associated primarily with increased knee cartilage defects and also with BMLs and knee symptoms in cross-sectional and longitudinal analyses, suggesting the abnormalities represented by these signals have a potentially important role in OA progression.

Chapter 6 illustrates the associations between quantitative measures of IPFP high signal intensity alteration and knee structural abnormalities in patients with symptomatic knee OA. 261 participants (mean age 63.0 ± 7.2 years) with symptomatic knee OA were selected from a randomized controlled trial with a follow up of 2 years. IPFP signal intensity alterations at baseline were quantitatively measured on T2-weighted fat-saturated MRI using MATLAB. These quantitative measures included the standard deviation [sDev (IPFP)] of whole IPFP signal intensity, the upper quartile value [UQ (H)] of high signal intensity, the ratio of volume of high signal intensity alteration to volume of whole IPFP [Percentage (H)] and Clustering factor (H) representing the clustering effect of high signal intensity. Cartilage volume and defects, and BMLs were assessed using validated measures. Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were associated with greater loss of tibial cartilage volume and larger increases in tibiofemoral cartilage defects over 2 years. Patients with high and medium tertiles of Clustering factor (H) had greater loss of cartilage volume compared with those with low tertile (4.9% and 4.6% vs 3.3% pa). Baseline Percentage (H) and Clustering factor (H) were positively and significantly associated with increases in tibiofemoral BMLs over 2 years. In conclusion, quantitative measures of increased signal intensity in the IPFP were associated with knee structural abnormalities longitudinally, suggesting that these measurements could be used as an additional entry criteria in order to enrich for 'faster progressors' in studies of knee OA.

Chapter 7 describes the associations between serum levels of resistin, IPFP measures and other knee structural changes in patients with knee OA. Patients (n = 200) with symptomatic knee OA (mean 63.1 years, range 49-79, female 46.5%) participated in this study and all measures were performed at baseline and two years later. Serum levels of resistin were measured using enzyme-linked immunosorbent assay. IPFP high signal intensity alteration and effusion-

synovitis were measured from MRI to represent knee synovitis. Knee structural changes including cartilage volume, cartilage defects and BMLs were assessed by MRI semi-quantitatively or quantitatively. Multilevel mixed-effects liner regression or logistic regression analyses were used in the data analyses. Serum levels of resistin were significantly and positively associated with the percentage of IPFP high signal intensity alteration volume to whole IPFP volume [Percentage (H)] (β: 1.34, 95% CI: 0.38, 2.30) and IPFP high signal intensity clustering (as measured by Clustering Factor (H), β: 0.69, 95% CI: 0.0.07, 1.31), as well as the presence (OR: 1.06, 95% CI: 1.02, 1.10) and volume (β: 0.77, 95% CI: 0.01, 1.54) of effusion-synovitis in multivariable analyses. It was also significantly and positively associated with total scores of tibiofemoral cartilage defects (β: 1.92, 95% CI: 0.31, 3.52) and BMLs (β: 2.97, 95% CI: 0.79, 5.15) after adjustment for covariates. In conclusion, serum levels of resistin were significantly and positively associated with IPFP measures and other knee structural abnormalities, suggesting a potential role of resistin in knee OA.

Chapter 8 determines the natural history of IPFP high signal intensity alteration (quantitative measures) and factors affecting with its changes over 2 years. A total of 233 patients (mean 63.0 years, range 49-79, female 42.1%) with symptomatic knee OA participated in this study. IPFP high signal intensity alteration, cartilage volume and effusion-synovitis were measured quantitatively, while cartilage defects and BMLs were assessed semi-quantitatively, from MRI at baseline and at 2 years later. X-ray was used to assess radiographic OA (ROA) at baseline. Over 2 years, nearly 90% of participants were with either worsening or improving IPFP high signal intensity alterations. In multivariable analyses, baseline age, total ROA scores and effusion-synovitis volume were positively and significantly associated with changes in IPFP high signal intensity alterations, while baseline cartilage volume was negatively and significantly associated with changes in these signal intensity alterations. There were positive

and significant associations of changes in cartilage defects, BMLs and effusion synovitis with changes in these signal intensity alterations. In conclusion, IPFP high signal intensity alterations were not static in patients with knee OA. Changes of these signal intensity alterations were age-related, independent of BMI, predicted by radiographic OA, cartilage volume and effusion-synovitis, as well as associated with changes in neighboring knee structures, suggesting therapeutic interventions aiming at these changes could be considered in clinical practice.

Chapter 9 summarises the findings of the thesis and provides a number of potential directions for future research.

Chapter 1 Introduction

1.1 Overview of osteoarthritis

Osteoarthritis (OA) is the most prevalent form of arthritis, characterized by progressive deterioration of joint structures, and mainly affects diarthrodial joints such as the knee, hip and hand. To date, it has been considered as a disease of the whole joint, affecting not only articular cartilage but also subchondral bone, menisci, ligaments, muscles, capsule and synovium (Figure 1.1) [1]. It is a multifactorial condition with risk factors including genetics, aging, female sex, injury and obesity. As aging and obesity are two important risk factors for onset of knee OA, its societal burden increases with increasing prevalence of obesity and the ageing of community.

Clinical symptoms of OA include pain, joint stiffness and dysfunction, which lead to physical disability and impaired quality of life. Although routine radiography is considered as the gold diagnostic standard in clinical practice due to the ability of revealing joint space loss and osteophytes at the joint margin, this method provides only indirect measurements of cartilage thickness and it is an insensitive reflection of cartilage loss [2]. Imaging biomarkers assessed on magnetic resonance imaging (MRI) are developed in research and clinical fields. Currently, there is still no cure for it, and current therapeutic strategies are primarily aimed at symptom-relief and joint arthroplasty is needed at the end-stage of this disease. Therefore, new interventions aiming to retard the progression of OA are urgently required.

1.1.1 Epidemiology and socioeconomic burden

Due to variations in definitions (pathological, radiographic and clinical) and joint sites (hand, knee and hip), prevalence and incidence estimates for OA differ widely. The knee is the most common joint affected by OA [3]. About one-third of participants age ≥ 60 had radiographic OA [4]. The prevalence of knee OA increases with age and is more common in women than

men. The age-standardised prevalence of knee OA was 19.2% in adults aged ≥ 45 and 37.4% in adults aged ≥ 60, while rises as high as 40% in adults aged ≥ 70 [3, 4]. As not all radiographic OA is associated with clinical symptoms, the prevalence of symptomatic knee OA is lower than radiographic knee OA. The age-standardised prevalence of symptomatic knee OA was 4.9% in adults aged ≥ 26. Among subjects aged ≥ 45, the prevalence of symptomatic knee OA was around 6.7% - 16.7%. The gender-standardised prevalence of symptomatic knee OA was 15.6% in men and 30.5% in women in participants aged ≥ 55 [5].

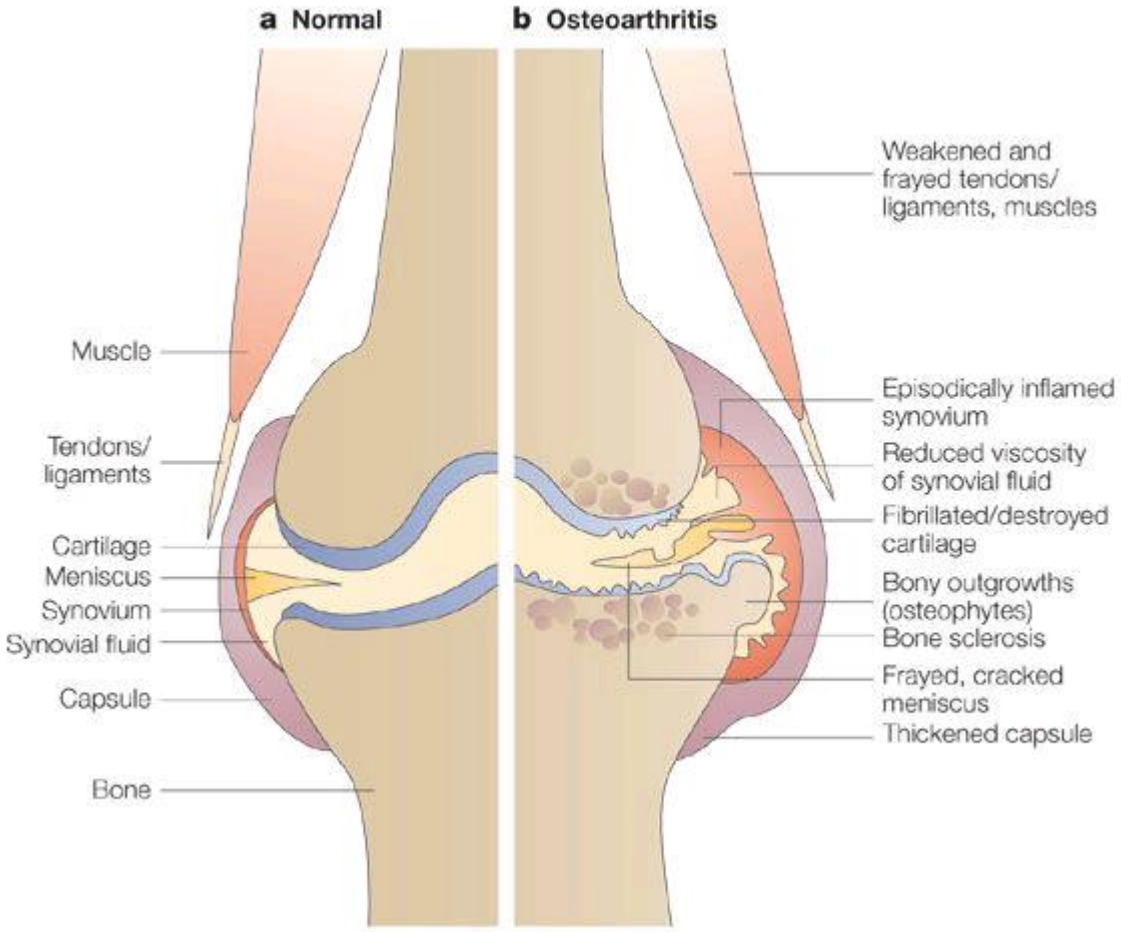

Figure 1.1 Pathogenic features consistent with osteoarthritis [6]

There is an insufficiency of relevant data on the cumulative incidence of developing OA. The incidence of radiographic knee OA was 2.5% per year in adults aged ≥ 55 [7], suggesting

radiographic OA is much common. Another study showed that the incidence of symptomatic knee, hip and hand OA were 6.5, 2.1 and 2.4 per 1000 person-year, respectively, lower than the incidence of radiographic OA [8]. However, recent systematic review described that incident radiographic OA was clinically relevant [9]. The incidence of OA is strongly associated with age and gender (Figure 1.2).

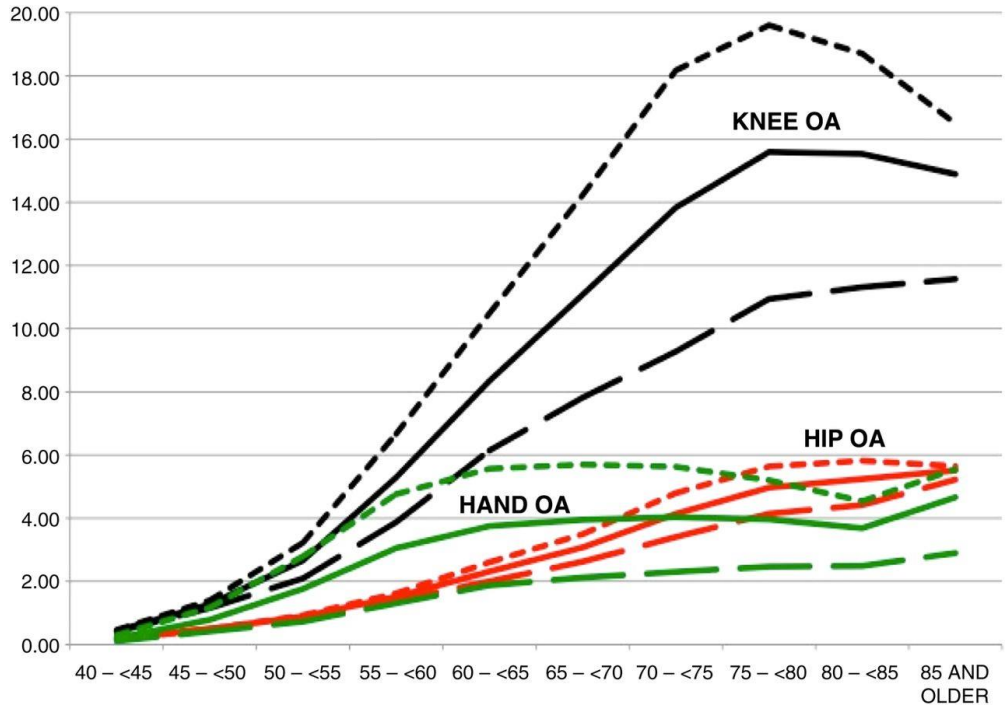

Figure 1.2 Age- and gender- specific incidence rates (/1000 person-years) of symptomatic osteoarthritis.
Solid: whole population; short dash line: females; long dash line: males. [10]

As OA has a high prevalence and incidence, socioeconomic burden of this disease should be urgently considered with increasing trends in population ageing, obesity prevalence and joint injury in the future. This burden is typically measured in direct, indirect and intangible costs [11]. The direct cost includes nonpharmacological treatments, pharmacological treatments, surgeries, adverse effects of treatment, long-term care and healthcare provision. In Western countries, the direct cost of OA has been estimated to account for between 1% and 2.5% of the gross national product of these countries [12]. A study estimated the direct cost of OA is

projected to increase from 2.9 billion to 7.6 billion Canadian dollars from 2010 to 2031 in Canada [13]. Absenteeism, reduced employment, reduced productivity, caregiver time and premature mortality constitute the indirect cost of OA, which could be up to eightfold greater than the direct cost and is hard to estimate [13]. The less-defined intangible cost such as pain, decreased quality of live, activity limitation and fatigue is also an important ingredient of total cost of OA [13]. Consequently, the true burden of OA is often underestimated and further studies are needed.

1.1.2 Pathology and pathogenesis

Knee OA is also characterized by progressive deterioration of joint structures, whereas, the specific structures such as meniscus and IPFP are involved in the process of this disease [14, 15].

The cartilage progressive degradation due to biomechanical and biochemical mechanisms has long been considered the crucial pathophysiological changes in knee OA process. However, the reasons underlying cartilage degradation are not yet well understood. Biomechanically, increasing focal stress through the joint caused by loss of cartilage in localized areas can lead to further cartilage loss, and along with the increasing cartilage loss or bony remodeling, the knee develops malalignment which increases further the degree of focal loading [16]. This feedback loop between increasing focal stress and cartilage loss could promote the progression of knee OA. The early stage of cartilage degradation is a result of failure of the repair process of damaged cartilage. This process is regulated by an interplay of anabolic and catabolic influences [3]. When this repair process exceeds the system’s ability to compensate as biochemical factors such as inflammation and metabolic changes contribute to this process,

this process becomes imbalance in favor of degradation [17]. Combining biomechanical and biochemical effects, cartilage degradation enters a vicious cycle.

1.1.3 Symptoms and signs

Pain is the hallmark clinical symptom of OA and the main reason for seeking medical advice. Joint pain is typically described as aggravated with activity and relieved with rest, while it may occur with rest in advanced disease and result in loss of sleep [18]. There is a discrepancy between radiographic joint abnormalities and reporting of pain in OA as there is a poor relationship between pain and radiographic OA [19]. Factors differentiating symptomatic OA from asymptomatic radiographic OA are largely unknown. One of these may be that radiographic measures have limited assessments of joint structure, especially the soft tissue. As cartilage is aneural, the pathogenesis of cartilage damage in OA cannot directly generate pain. The richly innervated joint structures such as subchondral bone, synovium, joint capsule, periosteum, ligaments and muscles could be the source of nociceptive stimuli in OA [20]. Patients with OA also experience stiffness, which is short-lived and occurs in the morning, after a period of inactivity, or particularly in the evening. This short-lived stiffness generally resolves in minutes and the duration is often less than 30 minutes differently from stiffness caused by rheumatoid arthritis [21]. Function impairment is another symptom reported by OA patients. Pain is the major cause of dysfunction, which includes poor mobility, limited daily activities, social isolation and loss of work opportunities [17]. Patients with OA may also complain of reduced movement, deformity, swelling and psychological distress [18].

The features on physical examination usually localise the symptomatic joint and vary with the severity of disease. The common signs of OA are list in Table 1.1 [18].

Table 1.1 Common signs of OA

Common signs of OA
Tenderness, usually located over the joint line
Crepitus with movement of the joint
Bony enlargement of the joint
Restricted joint range of motion
Pain on passive range of motion
Deformity
Instability of the joint
Altered gait
Muscle atrophy or weakness
Joint effusion

1.1.4 Risk factors

OA is a highly heterogeneous disease comprising various phenotypes that present with common clinical and pathological features [22]. The aetiology of this disease is not well understood, and recent evidence suggests it is a multifactorial and complex disease affected by person-level and joint-level risk factors [23]. Person-level risk factors, also can be called systemic risk factors, include ageing, female sex, obesity, inflammation and genetic factors, while joint-level risk factors, which alter the biomechanical stability of joint, are local factors including joint injury, joint structural abnormalities, synovitis and joint malalignment.

Ageing is one of the most prominent risk factors for the development of OA [24]. Although ageing and OA are closely linked, they are independent processes. Otherwise, numerous studies describe that the prevalence of radiographic and symptomatic knee, hip, hand and spine

OA increase with increasing age [25]. A review also described that the incidence rate of symptomatic OA peaked between the age of 50 and 70 [26]. A recent study conducted in Spain examined the incidence of clinically diagnosed OA and reported that female hand OA risk peaked between the age of 60 and 64, whereas knee and hip OA increased continuously with increasing age (Figure 1.2). The mechanistic understanding of how ageing contributes to the development of OA is still insufficient. The joint tissue changes with ageing together with other OA risk factors such as obesity, genetics and joint injury may contribute to the development of OA. The most plausible underlying mechanism may be age-related inflammation which increase production of pro-inflammatory mediators, the contributors of OA progression [27]. Other age-related factors that contribute to OA development include reduced muscle mass and increased fat mass, changes in the extracellular matrix, reduced cell density in the meniscus and ligaments, impairment in subchondral bone, as well as oxidative stress [28].

The prevalence of OA is more frequent among women than men at any given age of >50 years, with the sex difference most pronounced for hand and knee OA [23]. Women had higher incidence of clinically diagnosed OA in all joint sites (Figure 1.2). The Framingham study also described that women is an independent risk factor for knee OA [29]. This sex difference in both prevalence and incidence of OA increases with age may be related to endogenous oestrogen production and menopause, and further research is needed to reveal the mechanism in the future.

Obesity is one of the strongest predictors of OA development. An epidemiological study described that a high body mass index (BMI) was independently associated with knee and hand OA, but not hip OA [30]. A systematic review also suggested that obesity was the most consistent risk factor for onset of knee OA [31]. The underlying mechanism may include

abnormal joint loading and inflamed adipose tissue producing adipokines and inflammatory mediators which involve in cartilage degradation, synovial inflammation and bone erosions [32, 33] (Figure 1.3). Activated adipose tissue increases the synthesis of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α and IL-18, but decreases anti-inflammatory cytokines such as IL-10 [34]. Despite its ability of increasing pro-inflammatory cytokines, adipose tissue is a highly metabolic endocrine organ with the capacity to release adipokines such as leptin, resistin and adiponectin in spite of the fact that it is considered as a passive storage portal of energy [35]. These adipokines were detected in the synovial fluid and plasma of patients with OA [36, 37], and patients with OA had higher expression of adipokines than healthy controls [38]. Resistin has pro-inflammatory properties and the ability to trigger the release of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [39, 40], and its engagement with inflammatory conditions is rapidly investigated nowadays. Serum levels of resistin were correlated with increased synovial inflammation assessed histologically and positively associated with N-terminal propeptide of type III procollagen (sPIIINP) and the prevalence and incidence of radiographic knee OA [41, 42]. The synovial fluid levels of resistin were positively associated with local IL-6 levels in OA patients suggesting there may exhibit pro-inflammatory activity [43].

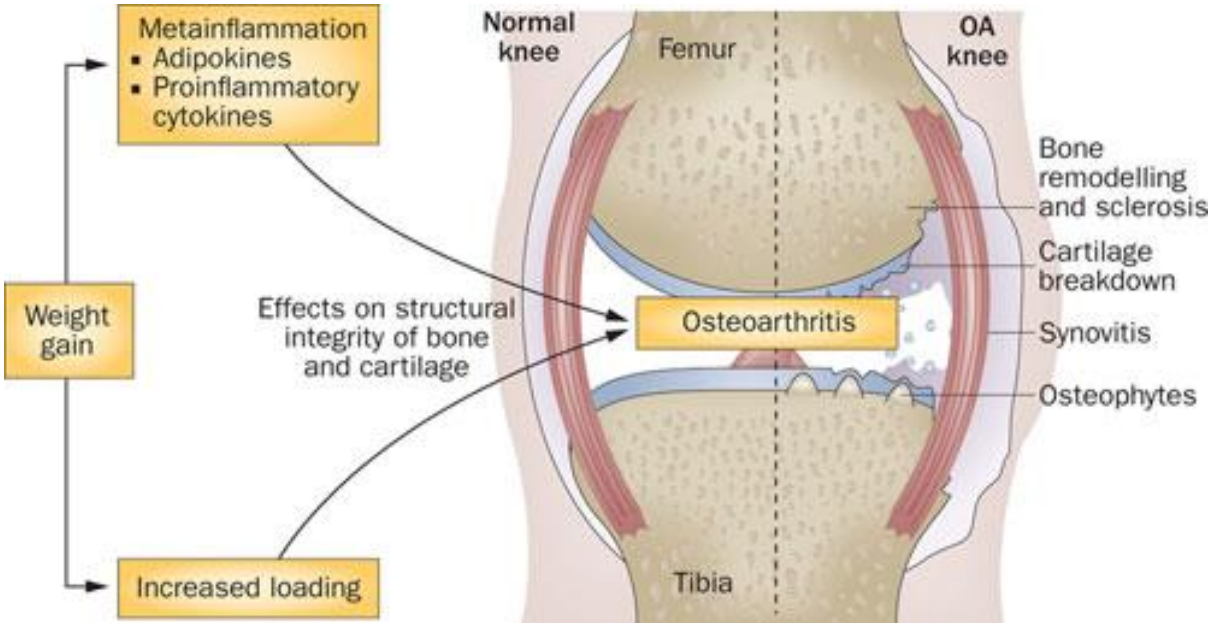

Figure 1.3 Pathogeneses of obesity in knee OA [44]

Joint-level risk factors for OA affects joint independently. While some joint-level risk factors may be shared by some joint, joint-level risk factors do not increase the risks of OA in all sites of the body. Previous joint injury is the main joint-level risk factor for OA. A systematic review suggested that a prior knee injury increased the risk of knee OA with pooled odd ratio (OR) of 2.83 [45]. A large longitudinal study also described that previous joint injury/surgery was an independent risk factor for hip and knee OA [46]. Joint injury can directly damage a number of joint structural tissues such as cartilage, ligaments and meniscus, which may primarily or secondarily contribute to the progression of OA. Joint shape abnormality is another central joint-level risk factor to OA pathogenesis of the joint, especially for hip OA. Overt congenital hip dysplasia, which generates progressive insufficient coverage of the femoral head by the acetabulum resulting in a concentrated weight-bearing area in the hip, is a well-recognized risk factor for hip OA [47]. Other joint-level risk factors such as local structural inflammation, joint malalignment, repetitive joint use through occupation and physical activity also contribute to the occurrence or progression of OA.

1.1.5 Diagnostic criteria

Routine radiography is considered as the gold diagnostic standard in clinical practice on grounds of the ability of revealing joint space loss and osteophytes at the joint margin. The radiographic diagnostic criteria for definition of knee OA were introduced in 1957 by Kellgren and Lawrence [48]. The Kellgren and Lawrence (K/L) grading system that is traditionally used to assess the severity of radiographic knee OA incorporates important radiographic features such as joint space narrowing (JSN) and osteophytes into one scale of increasing severity (Table 1.3). Although this grading system has limitations (e.g., its individual categories are non-isometric from each other) [49], it has been accepted as standard criteria by the World Health Organization in 1961 [50] as it is simple and less time consuming.

Although the predisposition to symptoms is related to the extent of radiographic features in OA patients, nearly half of patients with radiographic features of OA have no symptoms and vice versa [17]. Especially at the earliest stage of OA radiographs are insensitive to the pathological features, while with the progression of this disease radiographic features including JSN, osteophytes and subchondral bone abnormalities are visible. Hence, the absence of positive radiographic findings may not indicate the absence of symptomatic OA. Symptoms are usually the main cause that patients seek clinical help. Otherwise, OA has similar symptomatic features (i.e. pain) as other conditions such as avascular osteonecrosis, Paget's disease, complex regional pain syndrome, rheumatoid arthritis and stress fractures. Imaging measures including radiographs and MRI should be used to rule out other conditions. Thus, the diagnosis of the disease should rely on clinical and radiological features. American College of Rheumatology (ACR) radiological and clinical criteria for hand, knee and hip OA were developed, and widely used in clinical and research practice [21, 51-53]. The ACR criteria for hand, knee and hip OA are summarised in Table 1.2 [17].

Table 1.3 Kellgren and Lawrence (K/L) grading system

Definition grades	Description
Grade 0: Normal	No features of osteoarthritis
Grade 1: Doubtful	Possible osteophytic lipping and doubtful significance
Grade 2: Mild	Definite osteophytes and unimpaired joint space
Grade 3: Moderate	Moderate diminution of joint space
Grade 4: Severe	Joint space greatly impaired with sclerosis of subchondral bone

[54]

1.1.6 Treatment and management

As we described in Section 1.1.1, OA has high prevalence and severe socioeconomic burden. The aims of OA management include pain control, function improvement and disease modification [1]. Unfortunately, there are still no identified disease-modifying OA drugs (DMOADs) to modify structural progression. To date, several guidelines for the treatment or management of OA have been developed and the recommendations are often divided into three main categories such as non-pharmacological, pharmacological and surgical [55-60]. Depending on different phenotypes, risk factors, pathological mechanisms and affected sites, the modalities should be combined, or used individually.

Table 1.2 American College of Rheumatology (ACR) radiological and clinical criteria for hand, knee and hip OA

Site of joint	Criteria
Hand (clinical) *Diagnosis if 1, 2, 3, 4 or 1, 2, 3, 5 are*	1: Hand pain, aching, or stiffness for most days of previous month 2: Hard tissue enlargement of two or more of ten selected joints 3: Swelling in two or more metacarpophalangeal joints

present	4: Hard tissue enlargement of two or more distal interphalangeal joints 5: Deformity of two or more of ten selected joint
Hip (clinical and radiographic) *Diagnosis if 1, 2, 3 or 1, 2, 4 or 1, 3, 4 are present*	1: Hip pain for most days of previous month 2: Erythrocyte sedimentation rate of less than 20 mm in the first hour 3: Femoral or acetabular osteophytes on radiographs 4: Hip joint space narrowing on radiographs
Knee (clinical) *Diagnosis if 1, 2, 3,4 or 1, 2, 5 or 1, 4, 5 are present*	1: Knee pain for most days of previous month 2: Crepitus on active joint motion 3: Morning stiffness lasting 30 minutes or less 4: Age 38 years or older 5: Bony enlargement of the knee on examination
Knee (clinical and radiographic) *Diagnosis if 1, 2 or 1, 3, 5, 6 or 1, 4, 5, 6 are present*	1: Knee pain for most days of previous month 2: Osteophytes at joint margins on radiographs 3: Synovial fluid typical of OA (laboratory) 4: Age 40 years or older 5: Crepitus on active joint motion 6: Morning stiffness lasting 30 minutes or less

Non-pharmacological treatment

According to the ACR guideline 2012 [58], modalities are recommended strongly or conditionally for different sites of OA. Evaluation of the ability to perform activities of daily living (ADLs), instructions in joint protection and use of thermal modalities, assistive device and splints are recommended conditionally for hand OA. Aerobic and/or resistance land-based exercise, aquatic exercise and weight loss for obese patients are recommended strongly, while self-management programs, manual therapy, psychosocial interventions, appropriate instructions in joint protection and assistive device are recommended conditionally for knee OA. Recommendations for the management of hip OA are similar to those for knee OA. However, there are differences in anatomy and biomechanics between hip and knee joint, and hip and knee OA have different risk factors and various modalities for local treatments. The

effect size of a specific treatment may vary between hip and knee OA. Among the recommendations provided by ACR, European League Against Rheumatism (EULAR) and OA Research Society International (OARSI), education, change in lifestyles, exercise, and weight reduction are recommended consentaneously, and the non-pharmacological management of OA should be followed with a patient-centred, multidisciplinary approach rather than a discipline-specific approach [55, 58, 60].

Pharmacological treatment

The pharmacological treatment for OA include drugs for symptom relief such as acetaminophen, oral and topical nonsteroidal anti-inflammatory drugs (NSAIDs), tramadol and opioids, and for modifying the process of this disease such as strontium ranelate even they may have potential side effects and further randomised clinical trials (RCT) are needed to confirm their effects [61, 62]. Pharmacological treatment for OA can be applied site-specifically. Topical or oral NASAIDs and tramadol are recommended for hand, knee and hip OA, while acetaminophen and intraarticular corticosteroid injections are recommended for knee and hip OA but not hand OA [58]. Weak opioids and narcotic analgesics can be used to treat for refractory pain for hip and knee OA while other agents are ineffective or contraindicated; however, they are not recommended for hand OA [55, 58]. The toxicity or adverse events of pharmacological treatments should be considered in the clinical practice. For instance, in patients with risk factors of cardiovascular or gastrointestinal events, both non-selective NSAIDs and cyclo-oxygenase-2 (COX-2) selective drugs should be used cautiously.

Surgical treatment

Surgical interventions are generally the last choice for patients with advanced stage of OA while other no-surgical managements have failed [1]. The aims of surgical treatment are

symptom relief and function improvement to achieve better ADLs. OARSI guideline gives five surgical modalities for hip and knee OA: total joint arthroplasty, unicompartmental joint arthroplasty for knee OA, osteotomy and joint preserving surgical procedures, arthroscopic debridement and lavage in knee OA, and joint fusion when joint arthroplasty had failed [55]. There is still low level of evidence for hand OA in surgical treatments. EULAR guideline gives the recommendations for surgical management of hand OA based on low level of evidence, suggesting that interposition arthroplasty, osteotomy or arthrodesis are effective treatments for severe thumb base OA and should be considered when conservative managements are no longer effective [63]. The surgical modalities should be utilised based on the proper indications individually. For instance, arthroscopic debridement and lavage may improve the function for patients with loose bodies or flaps of meniscus or cartilage that causing mechanical symptoms, while osteotomy should be considered for patients with joint malalignment [55]. Joint arthroplasty is indicated for end-stage OA when conservative managements have failed [55].

1.2 Association between knee OA and IPFP

Knee OA is a disease driven by multiple risk factors and has been considered as a whole joint disease as it affects not only the articular cartilage and subchondral bone but also other structures of the joint, such as menisci, synovial membrane, joint capsule, ligaments, muscles and infrapatellar fat pad [1, 64]. Although the pathogeneses of knee OA is still unclear, biomechanical and biochemical pathways may both be involved in the incidence or progression of knee OA. IPFP, the local fat around the knee joint with an abundance of adipocytes, immune cells, vessels and nerve fibres [65], may have dual contributions to knee OA process. Biomechanically, IPFP may reduce the impact loading and absorb forces generated through the knee joint. Biochemically, it can produce both pro-inflammatory (deleterious) and anti-inflammatory (protective) cytokines such as interleukin (IL)- 1β, tumour necrosis factor (TNF)

α, IL-6, IL-8, basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF), as well as various adipokines such as leptin, resistin and adiponectin, all of which play roles in maintenance of cartilage and bone homeostasis [66-69]. An overview of the IPFP and its role in knee OA are included in this section.

1.2.1 Infrapatellar fat pad

IPFP, also known as Hoffa's fat pad, is an intracapsular but extrasynovial structure that locates in the anterior compartment of the knee [70]. It is richly vascularized and innervated, and may play an important role in anterior knee pain. As an important source of inflammation, IPFP may also be involved in the progression of knee OA [14]. This section reviews the literature of IPFP anatomy, histology, biomechanics, cells and secretory profiles.

1.2.1.1 Anatomy

IPFP is the biggest adipose structure in the anterior knee joint (Figure 1.4). It is situated beneath the patella, behind the patellar tendon and in front of the femoral condyles and tibial plateaus plateau [71]. As it is interposed between the joint capsule and the synovial membrane that lines its posterior aspect, it has been considered as an intracapsular but extrasynovial structure [70]. It attaches to the intercondylar notch of the femur by the plica ligamentum mucosum, as well as to the anterior cruciate ligament (ACL) in some subjects [72]. The normal IPFP also connects to transverse meniscal ligament, medial and lateral meniscal horns and retinaculum [72, 73]. It consists of a central body with medial and lateral extensions, occupying almost the whole anterior part of the knee [72]. Two clefts are typically present within the IPFP: one is vertical and located at the superior part of the IPFP and the other is horizontal and in a posteroinferior position [73].

It is vascularized by a rich anastomotic network. The supromedial and suprolateral geniculate arteries provide 2 vertical arteries and are linked horizontally with arteries running distally, while the central part of IPFP has low vascularization [74]. IPFP is also richly innervated, with an abundance of nerves from a branch of the tibial nerve arising from anterior fibbers of the popliteal plexus [75]. There were various nerves, such as a terminal branch of the obturator nerve, nerves to the vastus medialis and vastus lateralis, lateral articular and recurrent peroneal branches of common peroneal nerve, and the infrapatellar branch of the saphenous nerve, that also contribute to the innervation of the IPFP [75]. About one-quarter of the sensory fibres in the IPFP are nociceptive fibres targeted by substance P, which causes vasodilation that promotes the recruitment of immune cells [76]. As a richly vascularized and innervated structure in the knee, IPFP can be considered as an important source of knee pain and stiffness.

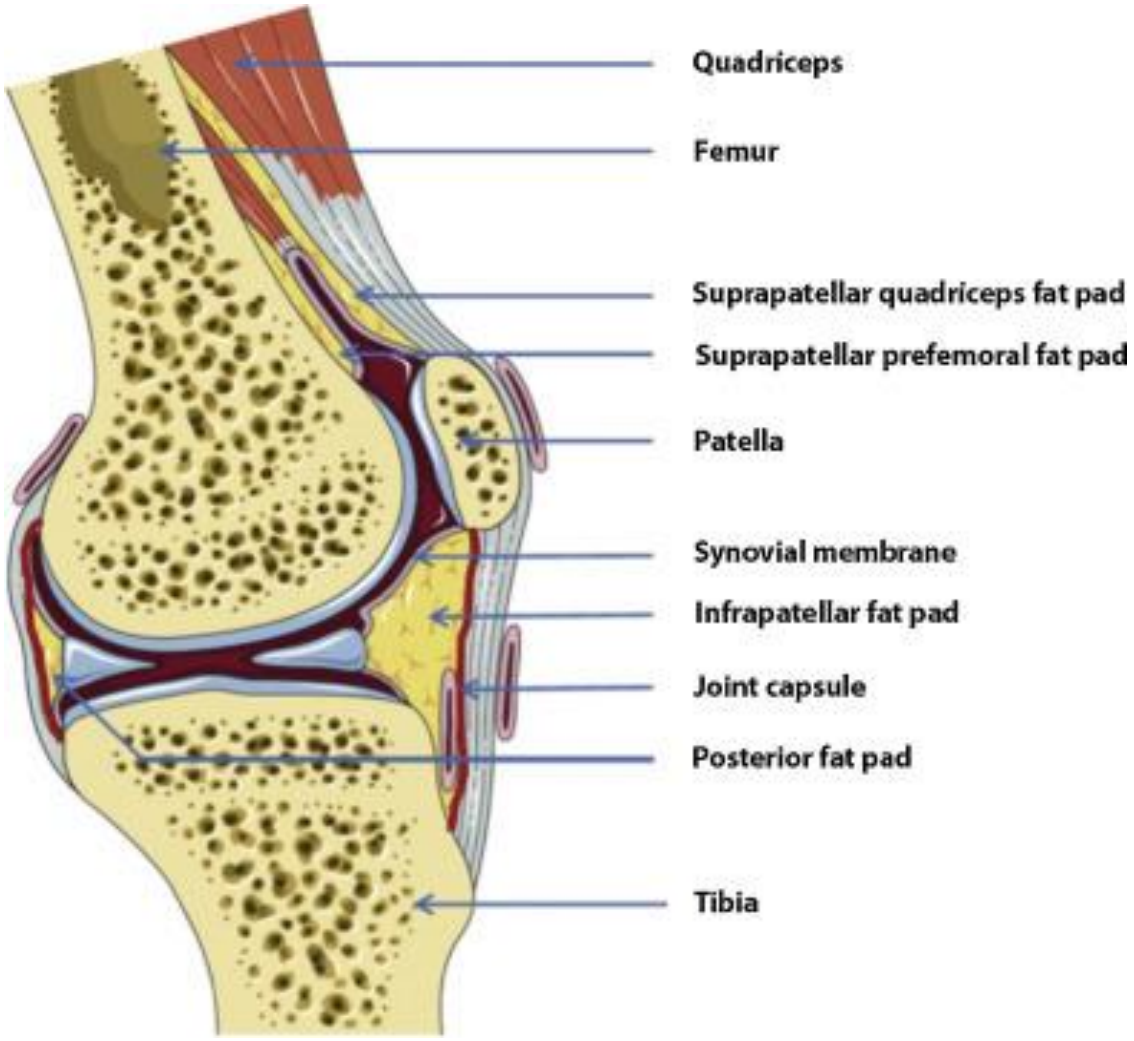

Figure 1.4 Anatomy of the infrapatellar fat pad [77]

1.2.1.2 Histology

The typical IPFP appears yellow and may also include lobules of fat extending from the surface, a superior segment extending posteriorly and frond-like projections of the synovium [78]. It is structurally similar to subcutaneous adipose tissue [79], comprising of a network of adipocytes, fibroblasts, leukocytes and collagen matrix. Otherwise, a recent histotopographic study shows that IPFP consists of white adipose tissue, of lobular type, with lobules delimited by thin connective septa, and the IPFP lobule area is smaller and the interlobular septa were thicker than subcutaneous adipose tissue of the abdomen, while the IPFP lobule area is lager and the interlobular septa were thinner than subcutaneous adipose tissue of the knee [80]. An animal

study also shows that IPFP contains two specific parts: inner tissue and outer tissue, which have different chemical compositions [81]. This study suggests that these two different tissues have different functions: the inner tissue is probably subjected to a compressive load, while the outer tissue is probably subjected to a tensional load. All these suggest that, unlike systemic adipose tissue, IPFP may have its specific functions in the knee joint.

1.2.1.3 Biomechanics

Along with the knee flexion, the angle between the patellar tendon and the anterior border of the tibia decreases, and the IPFP is extruded posteriorly as the space narrows [82]. While with the knee extension, it moves away from the anterior tibia [82]. Although the exact function of the IPFP is still not well understood, it may be involved in facilitating distribution of synovial fluid across the knee joint, providing lubrication [83, 84]. Furthermore, it probably provides shock absorbance from mechanical forces (similar to the menisci) and maintains knee stability [85-87]. As IPFP is an adaptable structure, its shape, position, pressure and volume vary considerably throughout the normal range of motion (ROM) of the knee joint [82, 86]. Thus, it could act as a plastic portion aiming to absorbing of pressure variations during knee joint activity [80].

1.2.1.4 Cell and secretory profiles

Adipose tissue was thought to act only as a reservoir for excess calories that are stored as triacylglycerol; whereas, it is believed that it could play an active role in physiologic and pathologic process in immunology and inflammation [88, 89]. As a specialized form of connective tissue, it contains a number of adipocytes, fibroblasts, macrophages, leukocytes, and other cells involved in inflammation. IPFP has the typical histological structure and the same cell profiles as adipose tissue [90]. The immune cells in IPFP can produce and release

inflammatory mediators, while adipocytes are responsible for the production of adipokines such as leptin, resistin and adiponectin [88]. Furthermore, it can secrete lipids (i.e. fatty acids) in the process of lipolysis. The details of each secretory profiles and their associations with knee OA will be discussed in Section 1.2.3.

1.2.2 IPFP and knee OA

As described previously, IPFP is a large joint structure in the knee. Its histological features imply it may have important biomechanical functions in knee joint. The cell and secretory profiles of the IPFP indicate that it may be involved in the pathogenesis of knee joint disorders.

1.2.2.1 Biomechanics

As described in Section 1.2.1.3, IPFP may have the capacity of lubrication and shock absorbance, and maintaining joint stability. It may also act as a mechanoreceptor corresponding to a tridimensional connective mesh working in the proprioceptive regulation of the joint activity [80]. Thus, IPFP abnormalities may contribute to the initiation or progression of knee OA. A case-control study compared two groups with or without evidence of oedema in the superolateral aspect of the IPFP and found that there were significant differences in Insall-Salvati index, patellar translation and patellofemoral angle between groups, suggesting that IPFP oedema is associated with patellar maltracking [91]. Another study found similar results [92] indicating that superolateral abnormalities in the IPFP may be an important abnormal biomechanical factor for patellofemoral joint and may be related to cartilage damage in this site.

IPFP volume may be related to its biomechanical abilities and be associated with knee OA. Recent studies described opposite results due to the different study design, sample size and measurements. Our previous study assessed IPFP maximum area in 977 older adults and found that this maximum area was significantly and negatively associated with radiographic OA, cartilage defect and BMLs, and knee symptoms, while significantly and positively associated with tibial and patellar cartilage volume cross-sectionally [93]. After an approximately 2.6 years follow-up in this study, we found that IPFP maximum area was beneficially associated with changes in knee symptoms, tibial cartilage volume and defects in women [94]. Using similar measurements in 297 adults without knee OA, Teichtahl et.al. reported that larger IPFP maximum area was associated with reduced knee pain, less cartilage volume loss after an average 2.3 years follow-up [95]. Another study measured the IPFP volume in 174 patients with knee OA and reported that greater IPFP volume was associated with greater cartilage volume, fewer cartilage defects, and higher presence of BMLs and osteophytes [96]. One case-control study showed that IPFP volume was lower in the group with patellar cartilage defects than that without [97]. In contrast, one cross-sectional study compared IPFP volume between participants with (n = 35) and without (n = 11) patellofemoral joint OA and reported that IPFP volume was greater in group with OA and associated with worse pain [98]. Variations in sample size, measurements and disease severity could explain the inconsistent results. At early stage of knee OA, IPFP volume may be increased as inflammation would induce hypertrophy of adipocytes and immune cells. At end stage of knee OA, IPFP volume may be reduced due to the necrosis and fibrosis. Further well-designed studies are needed for determining the associations between IPFP volume and knee OA.

1.2.2.2 Inflammatory cytokines

One underlying link between IPFP and knee OA may be the inflammatory cytokines. Although the trigger of IPFP inflammation is still unclear, it might be induced by obesity [99]. Figure 1.5 shows that there are more macrophages that are infiltrated in inflamed IPFP induced by obesity compared to unaffected IPFP. Another study described that inflammatory infiltrations were present within the IPFP in 36% patients with end-stage of knee OA [100]. Some studies have shown that the infiltration of immune cells in the synovium could contribute to the process of OA disease by the production of pro-inflammatory and/or pro-fibrotic cytokines [101-103]. Inflamed IPFP with a high infiltration of immune cells may also play important roles in OA progression. The high infiltrating immune cells in IPFP may be due to the high rates of nerve fibres staining positive for substance P [76] that has the ability of causing vasodilation leading to extravasation of immune cells.

In the pathogenesis of knee OA, the probable neurophysiological approach may elevate the release of substance P that activates related nerve fibres, generating a high infiltration of immune cells in IPFP. This high infiltration of immune cells within IPFP may affect knee OA processes by the production of pro-inflammatory cytokines which play roles in maintenance of cartilage and bone homeostasis [66-69]. The details of IPFP inflammatory characteristics have been investigated during the past decades. Ushiyama et al. reported that IPFP homogenates from knee OA patients contained detectable levels of IL-6 and TNF-α, suggesting that IPFP may be another source of these cytokines which may contribute to knee OA [104]. Another group described that IPFP tissues obtained from obese OA patients had a 2-fold increase in the expression of gene for IL-6 and in the release of IL-6, and a 3.6-fold increase in the release of soluble IL-6 receptor (sIL-6R) compared with subcutaneous adipose tissue (ScAT) [67]. Klein-Wieringa et al. also reported that IPFP samples from knee OA patients secreted higher levels

of IL-6, and larger amounts of TNF-α and adipokines were found in IPFP-conditioned media, compared with ScAT [66]. A recent study reported that autologous fibroblast-like synoviocytes from knee OA patients cultured in IPFP-conditioned medium had significant higher expression and release of pro-inflammatory cytokines than ScAT-conditioned medium, suggesting that IPFP may induce synovial inflammation in patients with severe knee OA [69]. The inflammatory process in IPFP may have a positive feedback. Clockaerts et al. found that the expression and release of inflammatory cytokines could be stimulated by IL-1β and inhibited by an agonist of peroxisome proliferator activated receptor α (PPARα) [68]. Witonski et al. investigated the association between IL-6 and TNF-α expression in IPFP and anterior knee pain and reported that patients with this knee pain had a significant higher expression of IL-6 and TNF-α in IPFP as compared to those who had no symptoms [105]. These suggest that IPFP may be involved in the pathogenesis of OA though inflammatory pathways.

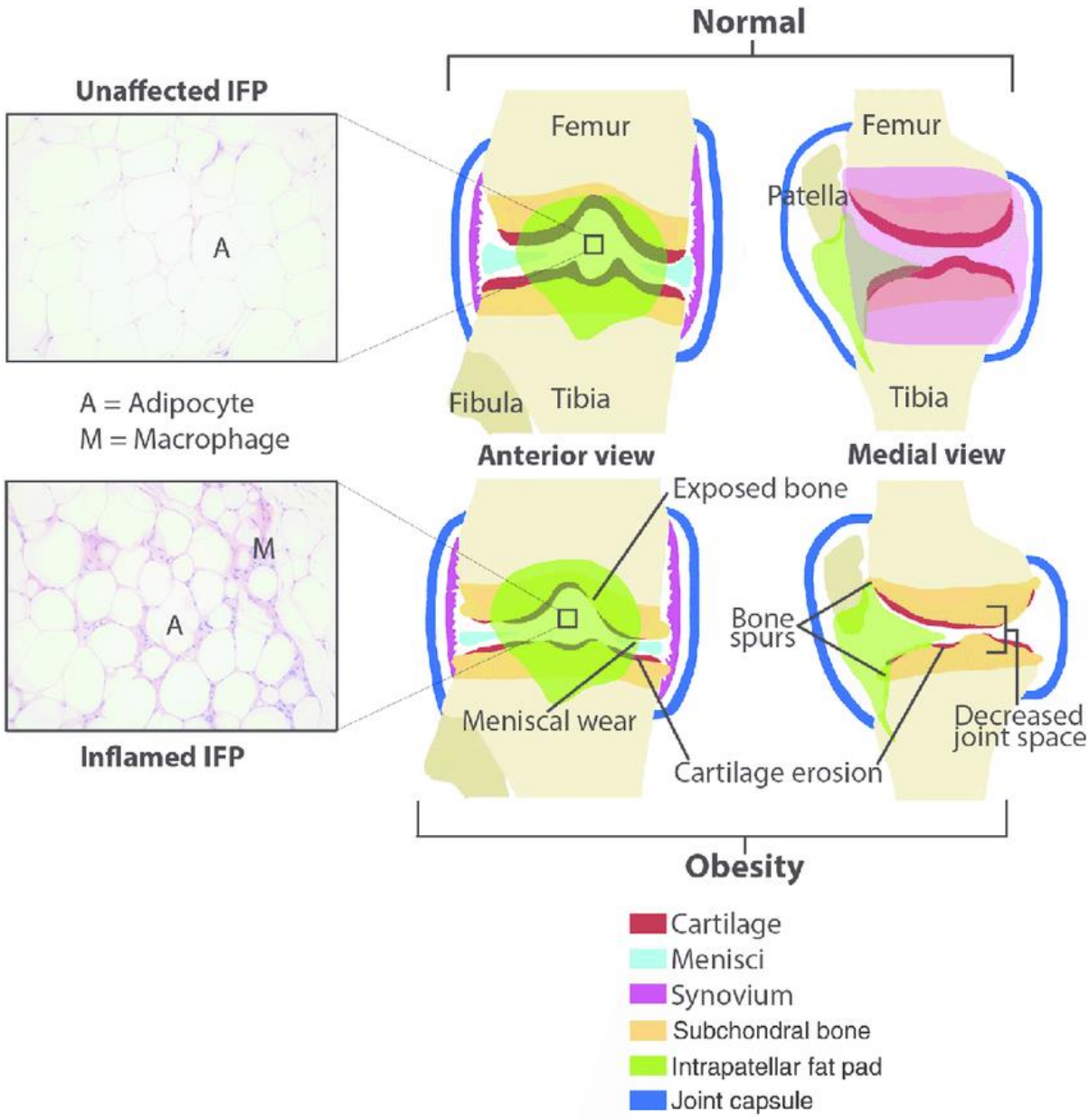

Figure 1.5 Histomicrographs of IPFP under normal conditions where OA is not present (top) vs. OA induced by obesity (bottom) [106]

1.2.2.3 Adipokines

Besides the cytokines such as IL-1, IL-6 and TNF-α secreted by IPFP, another group of proteins secreted mainly by white adipose tissues (also include IPFP) are adipokines, that can be also involved in the pathogenesis of knee OA. The adipokines act in an autocrine or paracrine manner and have potential roles in cartilage degradation, osteophyte formation and synovitis [107]. Leptin, well-researched adipokine, was thought to be beneficial for OA, as it

could upregulate proteoglycan synthesis and production of growth factors, and stimulate its own synthesis [36]. Recent evidence suggests that it has a different role. It can stimulate IL-1β production [108], increase the effect of pro-inflammatory cytokines [109], induce the expression of matrix metalloproteinases (MMPs) in OA cartilage [108, 110], activate nitric oxide synthase synergistically with interferon-γ or enhance activation of nitric oxide synthase 2 by IL-1 [109, 111], facilitate the activation of macrophages, neutrophils, natural killer cells and T helper 1 cells [112]. Another well-researched adipokine is adiponectin, which may have dual effects: it may have roles of resisting obesity and vascular diseases, and act as pro-inflammatory agent [113]. It can also induce production of IL-6, nitric oxide and MMPs in different sites in knee joint [114, 115]. Resistin has been described as an inflammatory factor being associated with multiple inflammatory diseases [116, 117]. This adipokine can act as an endogenous ligand of Toll-like receptor (TLR)-4 that triggers major inflammatory pathways and has a significant role in mediating inflammatory responses [118]. Recent studies described that resistin could induce the secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [39, 40] which had been implicated in the initiation and progression of OA [119].

The concentration of adipokines in the synovial fluid differs from serum levels, and joint structure may also release adipokines [120]. Synovium and osteophytes were believed to be the major sources of these adipokines [120]. Structurally similar as white adipose tissue, IPFP may also contribute to the release of adipokines. Previous studies have identified this secretory ability of IPFP [66, 68, 120, 121]. A recent study described that IPFP and synovium could express similar levels of adipokines, including leptin, chemerin and visfatin, in which leptin was the majority [121]. Interestingly, IPFP and synovium obtained from OA patients expressed significantly higher leptin and chemerin than those obtained from healthy donors. A similar result was found in another study, that IPFP from end-stage OA had higher expression of

adiponectin and leptin than those from early-stage of OA patients [122]. Although IPFP is not the only joint tissue secreting adipokines, the evidence shows that IPFP from OA patients releases higher amounts of adipokines, suggesting that it may be one pathway which IPFP involves in knee OA process.

1.2.2.4 Lipid mediators

Adipose tissue has the main biological function that stores and releases energy. Thus, it can secrete lipids that may also be involved in the process of knee OA. The main lipids and fatty acids are released in the process of lipolysis. These main lipids are considered to display immune modulatory properties: saturated fatty acids which have the pro-inflammatory effects [123], and unsaturated fatty acids which have the anti-inflammatory effects [124]. Besides fatty acids, their oxygenated derivatives, oxylipids, also have dual immune modulatory properties: anti- or pro- inflammatory effects, depending on their fatty acid precursor and the oxidizing enzymes involved in their synthesis [125, 126]. Prostaglandin E_2 (PGE_2), a mostly researched oxylipid, is produced by oxidation of ω-6 fatty acids by the cyclooxygenase, and has been shown to have a link with the pathogenesis of OA [127, 128]. Leukotriene B_4 (LTB_4), which has the same fatty acid precursor but is produced by the different oxidizing enzyme, the lipoxygenase, has the similar properties as PGE_2: regulating pro-inflammatory cytokines and interstitial collagenase synthesis. Interestingly, OA osteoblasts which produce high levels of PGE_2, have a low production of LTB_4 and vice versa [129], suggesting that there could exist a selective metabolism of fatty acid via different oxidizing enzymes in the process of OA.

IPFP may also release free fatty acids and oxylipids. A study cultured IPFP-derived adipocytes to obtain adipocytes-conditioned medium and reported that free fatty acids were present including oleic, linoleic, palmitic and palmitoleic acids, as well as monohydroxylated derivatives of arachidonic acid, 5-, 12- and 15- hydroxyeicosatetraenoic acid, and PGE_2 which were significantly detectable [130]. Another study compared different secretive profiles of fatty acids and oxylipids by IPFP obtained from OA and healthy donors [131]. It reported that a total of 29 oxylipids and fatty acids could be detected in IPFP-conditioned medium, and OA group contained reduced levels of the anti-inflammatory lipid mediator lipoxin A4 and elevated levels of thromboxane B2 and arachidonic acid compared with the healthy group. These interesting findings indicate that IPFP in OA patients may release more pro-inflammatory and less anti-inflammatory lipid mediators, which may be a pathway that IPFP contributes to OA pathogenesis.

In summary, IPFP may have dual roles in the homeostasis of the knee joint. With the normal structure and uninflamed status, IPFP may have the capacity of lubrication, pressure-relieving and shock absorbance, and joint stability maintenance, and could release anti-inflammatory cytokines, adipokines and lipid mediators. In contrast, inflamed IPFP may release pro-inflammatory cytokines, adipokines and lipid mediators. IPFP may play an important role in the initiation and progression of knee OA through biomechanical and inflammatory pathways.

1.3 MRI structural biomarkers

Radiographic OA including measures of JSN and osteophytes is an indirect measurement of cartilage loss [2]. It is weakly associated with clinical symptoms and a poor predictor of cartilage loss and total knee arthroplasty [132-134]. An estimate of over 10% of knee cartilage has been lost and over 40% patients have had cartilage defects at the time of knee structural changes presenting on a radiograph [135], suggesting it is not sensitive to detect structural changes in the knee. Plain radiography is not specific to one tissue. A recent study described that radiographic changes had no correlation with cartilage volume loss, but had a moderate correlation with changes in meniscal tears, over 10 years [136]. Thus, cartilage volume loss and meniscal pathologies cannot be distinguished on knee radiograph[137].

As knee OA has been considered as a whole joint disease, new methods which can assess most knee joint structural changes and are more sensitive to these changes are needed in research areas and clinical practice. Imaging biomarkers, especially acquired from MRI, have been used in OA research for a decade [133]. Because of its advantage in quantitative assessments of morphology and integrity of the whole joint, MRI has been considered a sensitive technique to assess cartilage and subchondral bone abnormalities, synovitis, ligament and menisci failures, and several semi-quantitative scoring systems have been developed for evaluating these structural changes in OA [138-140].

1.3.1 Cartilage volume

Traditionally cartilage loss was considered as the primary feature of knee OA progression. Although cartilage volume may increase due to swelling at an early stage of OA, cartilage volume is lost at a rate of up to 5% per year and 60% is lost by end stage knee OA [132].

Cartilage of the whole joint can be directly visualized on MRI in one examination. Thus, cartilage volume, regardless of its location, can be directly measured on MRI, and semi-automatically measures of the whole cartilage volume have been developed [141, 142]. Studies show that MRI-based cartilage volume measures have high accuracy and reproducibility [141, 143, 144]. These quantitative data could be beneficial in both the assessment of risk factors and longitudinal monitoring of OA.

The association between cartilage volume and knee symptoms is still inconsistent. A study including 132 subjects with symptomatic knee OA found a weak association between tibial cartilage volume and symptoms at baseline. In a longitudinal analysis, an increase in cartilage loss was weakly associated with worsening of symptoms [145]. Another study also reported that knee pain change was associated with a greater cartilage volume loss in the central tibial plateau [146]. In contrast, a study with a small sample size reported that changes in knee pain was not associated with cartilage loss over 2.4 years [147].

Cartilage volume is correlated with radiographic assessment. A cross-sectional study reported that radiographic joint space narrowing was inversely associated with tibial cartilage volume [148]. The same result was found in another study, that joint space narrowing but not osteophytes was associated with tibial cartilage volume [149]. A longitudinal study reported that joint space narrowing was strongly associated with cartilage loss in the central areas of both plateaus and condyles [146]. On the other hand, there are studies reporting no longitudinal associations between cartilage volume loss and radiographic changes in the knee [150, 151].

Cartilage volume loss is an independent predictor of total knee arthroplasty (TKA). One study reported that for every 1% increase in the rate of tibial cartilage loss there was a 20% increased

risk of undergoing a TKA after 4 years [132]. Another study reported that medial compartment of cartilage volume loss was one of strong predictors (OR: 18.70; 95%CI: 2.40 to 145.67) of TKA [152].

1.3.2 Cartilage defects

Cartilage defects, semi-quantitatively assessed by MRI, are the abnormal intrachondral signal or irregularities on the surface or bottom of articular cartilage [135]. This assessment can be used to evaluate the morphological characteristics of regional articular cartilage, while cartilage volume mainly represents global cartilage morphology. Although knee cartilage defects are very common in healthy individuals and have a relatively variable natural history [153, 154], improvement is highly unlikely and predicted by young age, lower BMI, decrease in BMI and no radiographic changes [135]. The etiology of cartilage defects remains unclear and they are not synonymous with cartilage loss. They can identify individuals at risk of faster cartilage loss, and can be regarded as important MRI biomarkers of early cartilage damage [133].

The associations between cartilage defects and knee symptoms have been investigated in the past decade. Most studies reported that the severity of cartilage defects was associated with knee pain in both young and older adults [135, 155-157]. One study investigated the associations between cartilage defects and incident knee pain, and found that medial tibial cartilage defects at baseline were positively correlated with the incidence of knee pain, as well as there was a positive association between progressive cartilage defects and the incidence of knee pain [158]. Another study also reported that cartilage defects could predict the increase of knee pain over 3.3 and 7.5 years[159]. Moderate to severe cartilage defects, especially full-

thickness cartilage defects accompanied by subchondral bone exposure, were more likely associated with knee pain [160].

Cartilage defects have been shown to have a significant association with radiographic changes in knee joint [161-163]. A cross-sectional study including 372 participants indicated that cartilage defects had an independent correlation with osteophytes, but not with joint space narrowing [153]. A longitudinal study with a 2.9-years follow-up showed that radiographic OA score was positively associated with cartilage defects cross-sectionally and could predict an increase in cartilage defects over years [154]. The same results were also found in another study [164].

Although cartilage defects are not synonymous with cartilage volume loss, there is a dose-response relationship between cartilage defect score and cartilage volume loss [165]. One study reported that patellar cartilage defects were positively associated with annual patellar cartilage loss, and this association was not found in tibiofemoral compartment [166]. Another study illustrated that baseline cartilage defects could predict cartilage volume loss at both tibial and patellar sites over 2.9 years [154]. Conversely, baseline cartilage volume had a site-specifically association with changes in cartilage defects over 2 years [167].

Cartilage defects are predictive of TKA. Subjects with higher score of cartilage defects were associated with a 6.0-fold increased risk of TKA over 4 years compared with those with lower

score of cartilage defects [166]. Recent studies also described that baseline cartilage defects were associated with an increased risk of TKA over 5 years [154, 168].

1.3.3 Bone marrow lesions

BMLs, also known as bone marrow oedema, are characterized as non-cystic ill-defined subchondral areas of high signal intensity on T2-weighted or proton density-weighted fat-suppressed (FS) fast spin echo (FSE) or short tau inversion recovery (STIR) images [169]. These regional bone marrow signal intensity alterations on MRI were initially described by Wilson et al. for the MRI finding in patients with severe knee and hip pain without any specific radiographic changes [170]. Histological studies have shown that various pathological entities, but not only oedema, could exhibit the same pattern on MRI [171-173]. One histological study using samples taken from end-stage knee OA patients undergoing TKA identified that these abnormalities consisted of bone marrow necrosis, abnormal trabeculae, bone marrow fibrosis, bone marrow oedema, and bone marrow bleeding [174]. Thus, the term of BMLs, but not bone marrow oedema, has been more commonly accepted in the OA research community [139].

BMLs has been associated with knee symptoms. A cross-sectional study reported that BMLs were independently correlated with the presence of knee pain and large lesions appeared exclusively in patients with knee pain [175]. The cross-sectional association between BMLs and knee pain was confirmed both in patients with knee OA and in older adults [156, 160]. A study using Boston Leeds Osteoarthritis Score (BLOKS) to assess BMLs reported that maximal area of BMLs had a positive association with VAS pain [139]. A nested case-control study examined the correlation of the development of knee pain with enlarging BMLs, and illustrated that cases without knee pain at baseline but with knee pain at follow-up had significantly larger

increases in BMLs compared to controls [176]. Zhang et al. reported that changes in BMLs were significantly associated with fluctuations in knee pain in patients with knee OA [177]. A population-based cohort study showed that changes in BMLs size was associated with changes in knee pain in participants without established ROA [178]. Two systematic reviews provided evidence that BMLs had a moderate to strong correlation with knee pain [179, 180].

BMLs could be considered as a predictor of radiographic progression of knee OA. One study with 15-month and 30-month follow-up investigated the association between BMLs and radiographic progression of knee OA, and found that BMLs were associated with site-specific progression of knee OA [181]. Another study defined the radiographic progression of knee OA as an increase of K/L score from ≤1 to ≥2, and reported that BMLs was positively associated with the progression of knee OA (OR: 2.0, 95%CI: 1.2 to 3.4) [182]. The similar results were obtained from other two studies [183, 184].

BMLs are associated with other MRI biomarkers. Carnes et al. demonstrated a cross-sectional association between cartilage defects and BMLs [154]. This cross-sectional association was also found in younger adults [185]. In addition, longitudinal associations between BMLs and cartilage loss were illustrated [139]. A longitudinal study reported that patellar BMLs were associated with patellar cartilage volume and defects at baseline, and could predict changes in both cartilage volume and defects in patellar compartment [186]; furthermore, patellofemoral cartilage volume was negatively associated with patellofemoral BMLs and baseline patellofemoral cartilage defects were positively associated with an increase in patellofemoral BMLs [187]. Besides the patellofemoral compartment, BMLs could predict site-specific

cartilage defect progression and cartilage volume loss in a dose-response manner in the tibiofemoral compartment [188].

The predictive values of BMLs for TKA have been investigated in recent years. One study with a 3-year follow-up reported that subjects with BMLs had a higher risk of TKA than those without BMLs [189]. BMLs, especially in the medial compartment, were the strongest independent long-term predictor of TKA [152]. In subjects from the general population, but not knee OA patients, baseline BMLs were predictive of TKA [178]. Another study also reported that increases in BML score was correlated with the risk of TKA [190].

1.3.4 Effusion-synovitis

Effusion-synovitis refers MRI-detected joint effusion, which equals both inflamed synovium and synovial fluid, but not the definite synovitis, and it is present in 96.3% knees with effusion [191]. Non-enhanced MRI cannot differentiate synovitis and effusion [140]. However, due to the higher cost and chance of side effects of enhanced MRI, effusion-synovitis measured by non-enhanced MRI has been widely used in OA research community. Furthermore, effusion-synovitis detected on un-enhanced MRI had higher correlations and sensitivity with synovitis measured by enhanced MRI [192]. Effusion-synovitis could be visualised in different regions within the knee, including peri-patellar areas, intercondylar region and around the anterior and posterior cruciate ligament [191].

Our group assessed effusion-synovitis using semi-quantitative and quantitative methods [193, 194]. Effusion-synovitis assessed by semi-quantitative method was independently associated

with knee pain both cross-sectionally and longitudinally [195]. Quantitative and semi-quantitative assessments of baseline effusion-synovitis were associated with baseline MRI-detected structural changes including cartilage defects, cartilage volume and BMLs, and predicted the changes in MRI-detected structures, in population-based study [193, 194]. In patients with knee OA, baseline cartilage defects and JSN were associated with change in effusion-synovitis, and baseline effusion-synovitis predicted changes in cartilage volume [196]. In this study, effusion-synovitis had a positive association with cartilage defects and BMLs, and a negative association with cartilage volume in analyses using mix-effects models. Another longitudinal study reported that baseline effusion-synovitis was associated with an increased risk of cartilage loss [197].

There is strong evidence to show that effusion-synovitis is correlated with symptomatic and radiographic progression of knee OA [198-201]. A nested case-control study using K/L score to define the incidence of knee OA, and reported that participants with the presence of effusion-synovitis had a higher risk for the occurrence of incident ROA [201]. A similar result was found by another nested case-control study, which showed that the presence of effusion-synovitis could predict the radiographic OA incidence [200]. Other two nested case-control study described that more cases with knee OA progression experienced worsening in effusion-synovitis [198, 199].

There are a few studies demonstrating effusion-synovitis predicts TKA [202, 203]. A study reported that participants who underwent TKA were more likely to have effusion-synovitis at baseline, and have worsening effusion-synovitis over 12 months, compared to those did not

undergo TKA [202]. A more recent study reported that knees exhibited effusion-synovitis at two or more subregions had increased risks of TKA in the subsequent 12 months compared with knees that did not [203].

1.3.5 Infrapatellar fat pad signal intensity alteration

Signal intensity alterations of IPFP measured by MRI can be regarded as an imaging biomarker for knee OA. These signal intensity alterations include higher or lower signal changes. IPFP high signal intensity alteration assessed on unenhanced T2-weighted or proton density-weighted fat-suppressed MRI, has been widely used in the clinical research community of knee OA as a surrogate for peripatellar synovitis [204, 205]. The contrast-enhanced T1-weighted MRI, which is the ideal way to quantify knee synovitis, cannot become the routine method to assess knee synovitis, as the contrast injection is costly and may have side effects.

High signal intensity alteration of IPFP are named as Hoffa-synovitis. However, a pathological study illustrated that there were vascular neoformations, fibrosis, and chronic inflammation in IPFP obtained from end-stage OA patients [206]. Signal intensity alterations within the IPFP may not be only a surrogate for synovitis, but also other pathological changes which may be involved in the pathogenesis of knee OA. Although a recent study suggested that effusion-synovitis should be preferred over Hoffa-synovitis as a surrogate marker for synovial thickening [192], IPFP signal intensity alteration may not only represent synovitis, should be given an important consideration in knee OA research.

IPFP high signal intensity alteration are associated with knee pain in knee OA patients [207]. A most recent study reported that Hoffa-synovitis was significantly associated with WOMAC knee pain [208]. Consistently, changes in Hoffa-synovitis were strongly related to changes in knee pain in longitudinal study [205].

IPFP high signal intensity alterations are associated with knee OA progression. A nested case-control study described that the presence of Hoffa-synovitis strongly predicted the development of radiographic knee OA [201]. Another nested case-control study showed that the presence of Hoffa-synovitis had strong associations with the increased risks of ROA in the subsequent 12 or 24 months [200]. Besides radiographic progression, two other nested case-control studies including symptomatic progression reported that cases experienced more worsening in Hoffa-synovitis than controls [198, 199].

IPFP high signal intensity alteration is related to MRI- and serum- biomarkers. A cross-sectional study described that high signal intensity alteration in superolateral IPFP was positively associated with cartilage damage and BMLs in patellofemoral joint [209]. In contrast, a study demonstrated that baseline Hoffa-synovitis was not associated with an increased risk of cartilage loss over 30 months [197]. There were also significant associations between Hoffa-synovitis and serum biomarkers including hyaluronic acid, MMP-3, and nitrated epitope of the α-helical region of type II collagen (sColl2-1NO2) [210]. Another study demonstrated that IPFP high signal intensity alteration was associated with serum IL-17 and adipokines such as adiponectin and resistin [211].

Similar as effusion-synovitis, Hoffa-synovitis can predict TKA [203]. Knees with Hoffa-synovitis in two or more subregions had the higher risk (OR: 2.17, 95%CI: 1.33 to 3.56) for TKA compared with knees that did not exhibit these features. Baseline Hoffa-synovitis had no associations with the risk of TKA, but the worsening of Hoffa-synovitis over 12 months was associated with an increased risk of TKA [202].

There are few studies reporting the associations between hypointense signals within IPFP and knee OA. Hypointense signals within IPFP on T2-weighted MRI may represent fibrosis or postoperative scarring [70, 73], chronic inflammation progressing from acute inflammation of the synovium due to micro-trauma of this tissue [212], or synovial thickening or fibrosis [213, 214]. A study compared low signal intensity changes in the plantar fat pad on MRI with histological changes and reported that these signal changes corresponded to fibrosis [215]. This fibrosis can be induced by chronic inflammation in the synovium [212], or periarticular surgeries or trauma around knees [70]. Although links between synovitis, surgical history and trauma in knee OA have been identified [216, 217], there has been no evidence showing that low signal intensity within the IPFP assessed by MRI is associated with symptoms and knee structure changes in the knee.

In summary, MRI biomarkers have been widely used in the clinical research of knee OA. New MRI biomarkers, or new methods to assess structural changes of the knee using MRI, is important in knee OA research. The thesis mainly focuses on the semi-quantitative and quantitative measurement of IPFP signal intensity alterations.

1.4 Summary

OA is the most common and prevalent chronic joint disorder in older adults, and the knee is the most common joint affected. To date, it has been considered as a disease of the whole joint, affecting not only articular cartilage but also subchondral bone, menisci, ligaments, muscles, capsule, synovium and IPFP. Although the aetiology and progression of this disease are not well understood, it is a multifactorial condition with risk factors including genetics, aging, female sex, injury and obesity.

IPFP may have dual roles in the homeostasis of knee joint. With the normal structure and uninflamed status, IPFP may have the capacity of lubrication, pressure-relieving and shock absorbance, and joint stability maintenance, which contributes to the normal function of the knee joint. In addition, unaffected IPFP may release anti-inflammatory cytokines, adipokines, and lipid mediators, which help keep the knee joint free of inflammation. After a trigger (e.e. obesity, trauma, or ageing) is pulled, IPFP becomes inflamed and releases more pro-inflammatory cytokines, adipokines, and lipid mediators, which cross-talks with other joint structures such as the synovium, cartilage, meniscus, and subchondral bone, and results in low-grade inflammation in the knee joint. This low-grade inflammation could induce the degradation of cartilage and other knee joint structures. Furthermore, high release of pro-inflammatory mediators may lead to knee pain and other symptoms. The degradation of the IPFP causes abnormal changes in knee structures and function. Abnormal IPFP may play an important role in the initiation and progression of knee OA through biomechanical and inflammatory pathways.

MRI is becoming a popular research tool for the visualization of all structural abnormalities within the knee joint. Besides the commonly used MRI biomarkers such as cartilage volume,

cartilage defects, BMLs and effusion-synovitis, MRI can also assess IPFP abnormalities semi-quantitatively and quantitatively, which may be correlated with disease symptoms and progression of knee OA.

The following chapters investigate the associations between semi-quantitative and quantitative IPFP high or low signal intensity alterations and joint symptoms, structural changes, and serum biomarkers in knee OA, as well as the natural history and factors affecting their changes. The research questions which directed this work are described in the following chapter.

Chapter 2 Research Questions

Chapter 1 reviews the background and rationale of this thesis. The research questions of this thesis are summarized as follows:

1. Is there any cross-sectional and/or longitudinal association between semi-quantitative measures of infrapatellar fat pad high signal intensity alteration and knee symptoms and structural changes in older adults?
2. Is there any cross-sectional and/or longitudinal association between semi-quantitative measures of hypointense signals in the infrapatellar fat pad and knee structural change and symptoms in older adults?
3. Are quantitative measures of infrapatellar fat pad high signal intensity alteration associated with knee structural abnormalities in patients with symptomatic knee OA?
4. Is serum levels of resistin associated with knee synovitis measures and structural changes in patients with knee OA?
5. What is the natural history of infrapatellar fat pad high signal intensity alteration (quantitative measures) and which factors could affect its changes?

Chapter 3 Methodology

Chapter 4 and 5 arose from analyses using the data from the TASOAC study. Chapter 6, 7 and 8 arose from analyses using data from the VIDEO study, which is a randomized, double-blind, placebo-controlled clinical trial. This chapter describes the study population and design for the TASOAC and VIDEO study, as well as the protocols for measurements of factors which are common to multiple chapters in the thesis. Additional factors which are unique to each chapter are described in more detail in the methodology section of each of the subsequent chapters.

It should be noted that the following chapters are presented in the form in which they were submitted to, or accepted by, peer-reviewed journals for publication. Thus, throughout these chapters, there are some differences in the description of methods, analyses, results, and interpretation, due chiefly to requests from journal reviewers.

3.1 Study Design of Tasmanian Older Adult Cohort (TASOAC) study

The TASOAC study is an ongoing prospective, population-based study, aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA (assessed by both X-ray and MRI) at multiple sites including hand, knee, hip and spine.

3.1.1 Study population and design

The cohort consisted of participants aged between 50 and 80 years (mean: 62 years; SD: 7 years), selected from the roll of electors in southern Tasmania (population 229,000), a comprehensive population listing with an equal number of men and women, using stratified simple random sampling without replacement. Due to the compulsion of voting in federal and state election in Australia, electoral rolls represent the complete population information. The sample was stratified by sex to provide equal numbers of men and women, and equal distribution was drawn from urban and rural areas in Southern Tasmania. Institutionalized older adults were excluded in this study as it was designed to examine community-dwelling older adults. Participants, who reported contraindication for MRI such as metal sutures, the presence of shrapnel, iron filings in the eye and claustrophobia, were excluded as this test was required in this study.

The overview of participant recruitment and withdrawal during the study period was shown in Figure 3.1. The first phase of this study was performed between March 2002 and September 2004. A total of 2,135 eligible participants were identified from which 1,904 were able to be contacted. Of them, 1,100 enrolled in the study, and 1099 attended a baseline clinic (response rate 51%). The phase 2 follow-up was conducted 2.6 years later (rang: 1.4 – 4.9 years), with a set of measures completed also at a phase 3 follow-up after 5.1 years (range: 3.6 – 6.9 years),

and a phase 4 follow-up after 10.7 years (range: 9.2 – 12.5 years).

3.1.2 Sample size

This study was in progress when the commencement of the PhD candidature, thus, formal sample size calculations were not performed during the design of the thesis. Participants in the analyses reported in the thesis were limited to those recruited at baseline and follow-up, and to those had completed data for relevant outcome and study factors. Therefore, sample sizes vary between chapters, and the reasons for exclusion are described in each chapter. Otherwise, it subsequently proved that sample sizes were more than adequate to answer the thesis research questions.

3.1.3 Ethical issues

The TASOAC study were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee (Ethics Approval Number: H6488). Written informed consent was obtained from all participants prior to enrolment in the study.

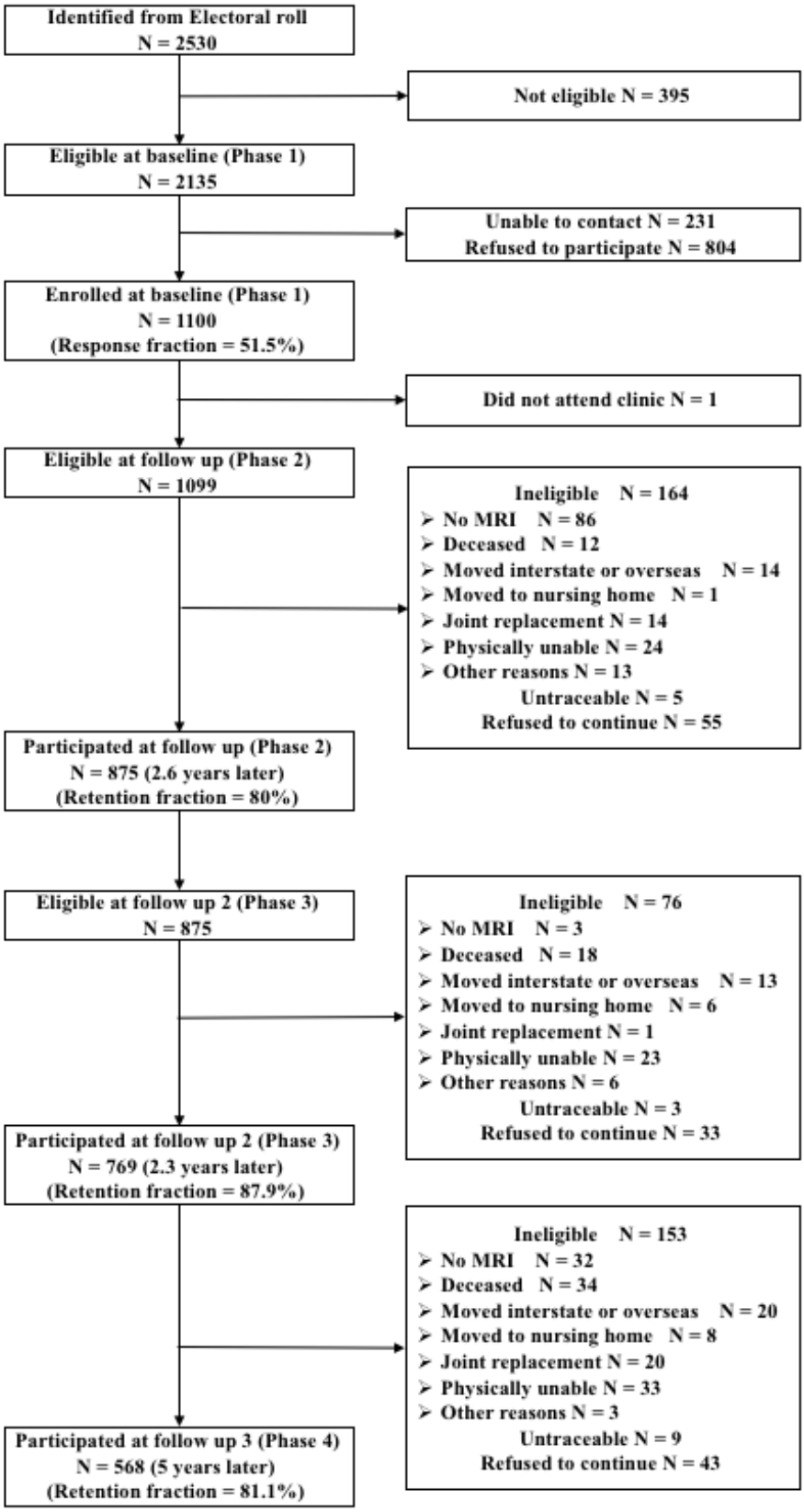

Figure 3.1 Flowchart of TASOAC study participants.

3.2 Study design of Vitamin D Effect on Osteoarthritis (VIDEO) study

VIDEO study is a multicentre, randomized, placebo-controlled double-blind clinical trial. The aim of this study is to compare the effects of vitamin D supplementation versus placebo on knee structural and symptomatic changes in patients with symptomatic knee OA over a 2-year period.

3.2.1 Study population and design

The VIDEO study recruited patients with symptomatic knee OA in Southern Tasmania and Melbourne, by using a combined strategy, including collaboration with general practitioners, specialist rheumatologists, and orthopaedic surgeons, as well as advertising through local media. The inclusion and exclusion criteria of participants for this study are listed below:

Inclusion criteria:

I. Age 50-79 years old;
II. Men and women with symptomatic knee OA for at least 6 months with a pain visual analogue scale (VAS) of at least 20 mm;
III. Meet the ACR criteria [21] for symptomatic knee OA assessed by a rheumatologist;
IV. Have an ACR functional class rating of I, II and III [218];
V. Have relatively good health (0-2 according to the investigators global assessment of disease status on a 5-point Likert scale, range 0 [very well] to 4 [very poor]); and
VI. Have serum vitamin D level of >12.5 nmol/L and <60 nmol/L.
VII. Is able to read, speak and understand English, capable of understanding the study requirements and willing to co-operate with the study instructions.

Exclusion criteria:

I. Patients with severe radiographic knee OA (grade 3 according to Altman's atlas [219]);

II. Patients with severe knee pain (on standing more than 80 mm on a 100-mm VAS);

III. Any contra-indication to having an MRI.

IV. Patients with rheumatoid arthritis, psoriatic arthritis, lupus, or cancer;

V. Patients with severe cardiac or renal function impairment

VI. Patients with hypersensitivity to vitamin D;

VII. Patients with any condition possibly affecting oral drug absorption (e.g. gastrectomy or clinically significant diabetic gastro-enteropathy);

VIII. Having significant trauma to the knees including arthroscopy or significant injury to ligaments or menisci of the knee within 1 year preceding the study;

IX. Having anticipated need for knee or hip surgery in the next 2 years;

X. Having taken Vitamin D supplements in last 30 days.

An overview of participant recruitment and withdrawal during this study is shown in Figure 3.2. A total of 599 participants were screened for eligibility from Jun 2010 to Dec 2011. Of which, 186 participants were excluded according to the exclusion criteria, and 413 participants were randomized to treatment and control groups.

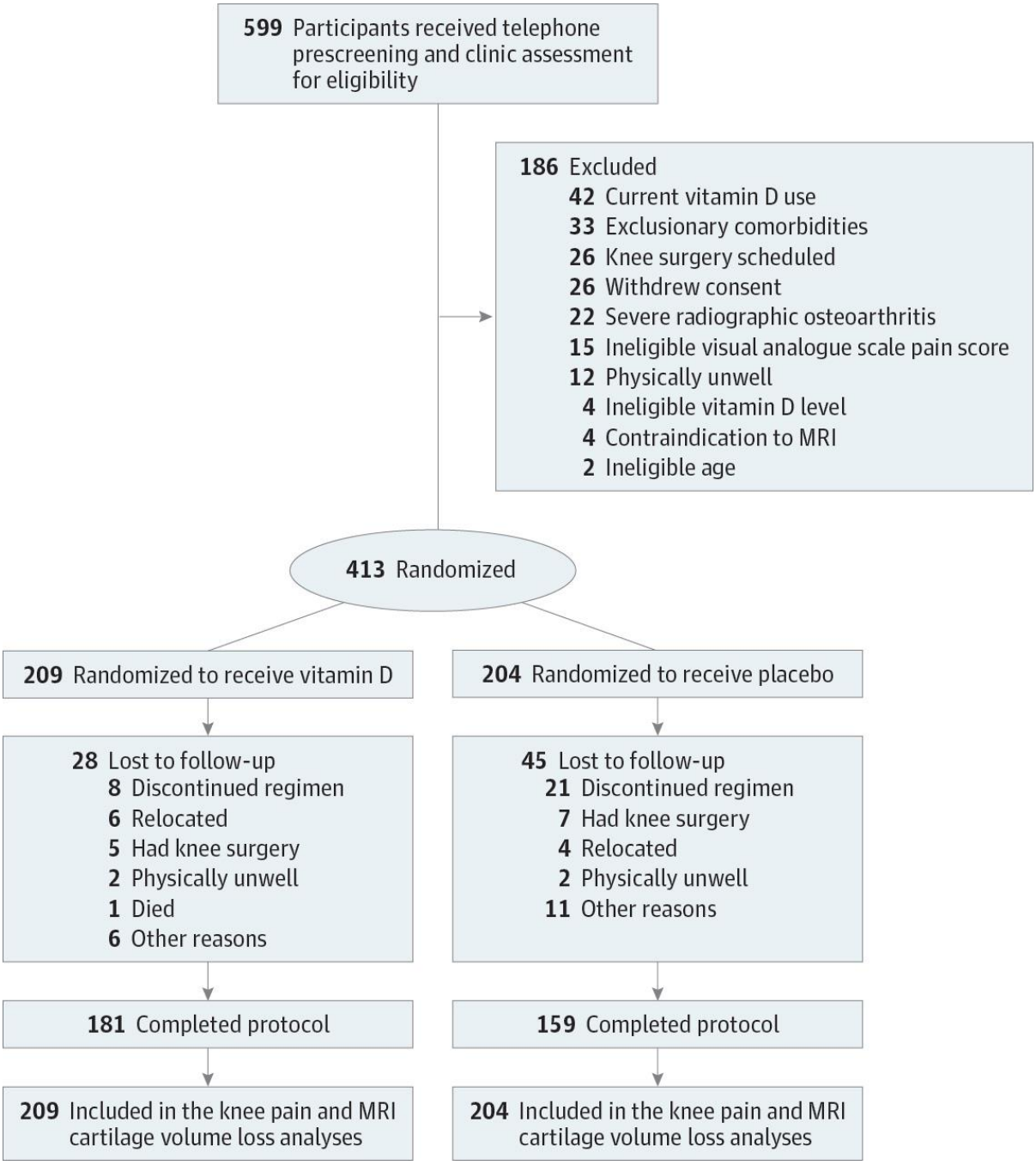

Figure 3.2 Flowchart of VIDEO study participants.

3.2.2 Randomization and masking

Participants were allocated to either vitamin D or placebo group at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a central automated allocation procedure that was independent of investigators. Participants, research coordinators and investigators were all blinded to the treatment assignment. Blinding was

maintained until all the data were collected, confirmed for accuracy and cleaned, and statistical analyses were performed.

3.2.3 Interventions

Participants in the treatment group were given a monthly capsule of 50,000IU (1.25 mg) vitamin D3 (cholecalciferol) for 24 months. The vitamin D3 compound was purchased from Nationwide Compounding Pharmacy, Melbourne, Australia. In the control group, participants were given an identical inert placebo provided by the same company.

3.2.4 25OHD assays

Serum 25OHD was assayed at screening, month 3 and 24, utilizing a direct competitive chemiluminescent immunoassay (DiaSorin Inc., Stillwater, Minnesota, USA). The intra- assay and interassay coefficients of variation were 3.2% and 6.0%.

3.2.5 Outcomes

Primary outcome measures were changes in knee pain assessed using the WOMAC score from baseline to month 24. Secondary outcomes included VAS knee pain, lower limb muscle strength and structural changes on MRI.

3.2.6 Sample size

Sample size calculation assumed $\alpha = 0.05$ and $\beta = 0.20$, and was performed based on the Cohen formula [220]. For change in WOMAC pain, the previous study reported a standard deviation

of 70.5 on a score from 0 to 500 [221]. The minimal clinically important difference (MCID) for WOMAC pain was previously reported to be 16% reduction of the score from baseline [222, 223]. With 400 participants, a difference between groups of 20 units on the score is detectable with 80% power.

3.2.7 Ethical issues

Ethics approval was received from the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616). Informed written consent was obtained from all participants.

3.3 Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) by using a single pair of electronic scales (Delta Model 707, Seca, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index (BMI, weight (kg)/height (m^2)) was calculated.

3.4 X-ray

A standing anteroposterior semi-flexed view of the right knee with 15 degrees of fixed knee flexion was performed, and radiographs were individually assessed for JSN and osteophytes on a scale of 0-3 (0 = normal and 3 = most severe) using the OARSI atlas developed by Altman et al [224]. JSN was assessed in medial and lateral compartments, while osteophytes in medial

femoral, medial tibial, lateral femoral, and lateral tibial compartments. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. Intra-observer repeatability was assessed in 40 subjects with an interval of at least one week between the two measurements. Intraclass correlation coefficients (ICCs) ranged from 0.65– 0.85. The presence of ROA was defined as any score ≥ 1 for JSN or osteophytes, as previously described [149].

3.5 Magnetic resonance imaging

3.5.1 MRI protocol

In the TASOAC study, MRI scans of the right knees were performed at baseline and follow-up. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH, USA) with use of a commercial transmit-receive extremity coil. Image sequences included the following: (1) a T1-weighted fat saturation three-dimensional (3D) gradient-recalled acquisition in the steady state, flip angle 30◦, repetition time 31 ms, echo time 6.71 ms, field of view 16 cm, 60 partitions, 512 × 512 pixel matrix, acquisition time 5 min 58 s, one acquisition; sagittal images were obtained at a slice thickness of 1.5 mm without an inter-slice gap; and (2) a T2-weighted fat saturation two- dimensional (2D) fast spin echo, flip angle 90◦, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228 × 256 pixel matrix; sagittal images were obtained at a slice thickness of 4 mm with an inter-slice gap of 0.5–1.0 mm.

In the VIDEO study, MRI scans of the study knee were obtained according to a standardized protocol using a 1.5 T whole-body MRI unit with a commercial transmit-receive extremity coil. The following sequences were used: a) sagittal fat saturated (FS) T1-weighted spoiled gradient

echo (GRE) with flip angle 30◦, repetition time 31 ms, echo time 6.71 ms, field of view (FOV) 160 mm, acquisition time 5 min 58 s, 60 slices, 512 × 512 pixel matrix slice thickness of 1.5 mm without between-slice gap. b) T2-weighted/proton density-weighted fast spin echo (FSE) sequences: sagittal FS T2-weighted 3D FSE sequence, flip angle 90◦, repetition time 3,067 ms, echo time 112 ms, FOV 16 cm, 15 slices, 228 × 256 pixel matrix slice thickness of 2 mm with a between-slices gap of 0.5–1.0 mm; coronal FS proton density- weighted FSE sequence, repetition time 3,400 ms, echo time 64 ms, flip angle 90◦, slice thickness 3 mm, FOV 16 cm, pixel matrix 256 × 256, acquisition time 5 min 26 s.

3.5.2 Cartilage volume assessment

Cartilage volume was assessed using the previously described image processing techniques [225]. The volumes of individual cartilage plates (medial tibial, lateral tibial and patellar) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX Lite imaging software (32-bit version 5·9, Pixmeo SARL, Geneva, Switzerland). The coefficient of variation (CV) was 2·1% for medial tibia and 2·2% for lateral tibia [226].

3.5.3 Cartilage defects assessment

Cartilage defects were evaluated using a modified Outerbridge classification [227] at medial tibial, lateral tibial, medial femoral, lateral femoral, femoral trochlear, and patellar sites: grade 0, normal cartilage; grade 1, focal blistering and intracrtilaginous hyperintensity with an normal contour; grade 2, irregularities on the surface and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50% without exposure of subchondral bone; grade 4, full thickness chondral wear with exposure of subchondral bone. Intra-observer

reliabilities (expressed as ICCs) ranged from 0.89 to 0.94 for different compartments. Inter-observer reliabilities were assessed in 50 MR images and yielded correlation coefficients of 0.85 to 0.93 for different compartments [153].

3.5.4 Subchondral bone marrow lesions assessment

Subchondral bone marrow lesions (BMLs) were defined as discrete areas of increased signal adjacent to the subchondral bone. They were measured semi-quantitatively using the modified Whole-Organ Magnetic Resonance Imaging Score (WORMS) method [138]. BMLs were scored from 0 to 3 based on the extent of subregional involvement (0 = none; 1 = < 25% of the subregion; 2 = 25-50%; 3 = >50%). The ICCs were 0.93-0.98 for this scoring system [228].

3.5.5 Effusion-synovitis assessment

Quantitative measurement:

Effusion-synovitis was distinguished in the following subregions according to the anatomy of the knee joint synovial cavity [229]: 1) the suprapatellar pouch, extending superiorly from the upper surface of the patellar, between the posterior suprapatellar fat pad (quadriceps femoris tendon) and the anterior surface of the femur; 2) the central portion, that includes the area between the central femoral and tibial condyles, around the ligaments and menisci, and the area behind the posterior portion of each femoral condyle, inside of the joint capsule. The volumes of individual joint subregions were isolated from the total volume by selecting each region of interest (ROI) according to the intra-articular fluid-equivalent signal on a section-by-section basis. The final 3-D volume rendering was generated using commercial in-house OsiriX Lite imaging software cursors (32-bit version 5.9, Pixmeo SARL, Geneva, Switzerland) [230]. The

readers were blinded to treatment allocation and patients' information. The ICCs were 0.96-0.97 and inter-reader correlation coefficients were 0.93-0.99 in different subregions.

Semi-quantitative measurement:

Effusion-synovitis in each subregion was scored individually according to WORMS, grading collectively from 0 to 3 based on the estimated maximal distention of the synovial cavity as following: 0 = normal; 1 = ≤ 33% of maximum potential distention; 2 = 33%-66% of maximum potential distention; 3 = ≥ 66% of maximum potential distention [138]. The inter-reader reliability was 0.63-0.75 and intra-reader reliability was 0.60-0.75 (weighted κ) in different subregions as described previously [231].

3.5.6 IPFP signal intensity alteration assessment

Semi-quantitative measurement:

IPFP signal intensity alteration at baseline and follow-up was assessed by an experienced orthopaedist trained by an experienced radiologist, using T2-weighted MR images (Figure 3.3). Signal intensity alteration, defined as discrete areas of increased signal within IPFP, was graded as follows: grade 0 = none; grade 1 = < 10% of the region; grade 2 = 10% to 20% of the region; grade 3 = >20% of the region. Intraobserver and interobsever reliabilities were assessed in 100 subjects with an ICCs of 0.90 and an interclass correlation coefficient of 0.89, respectively.

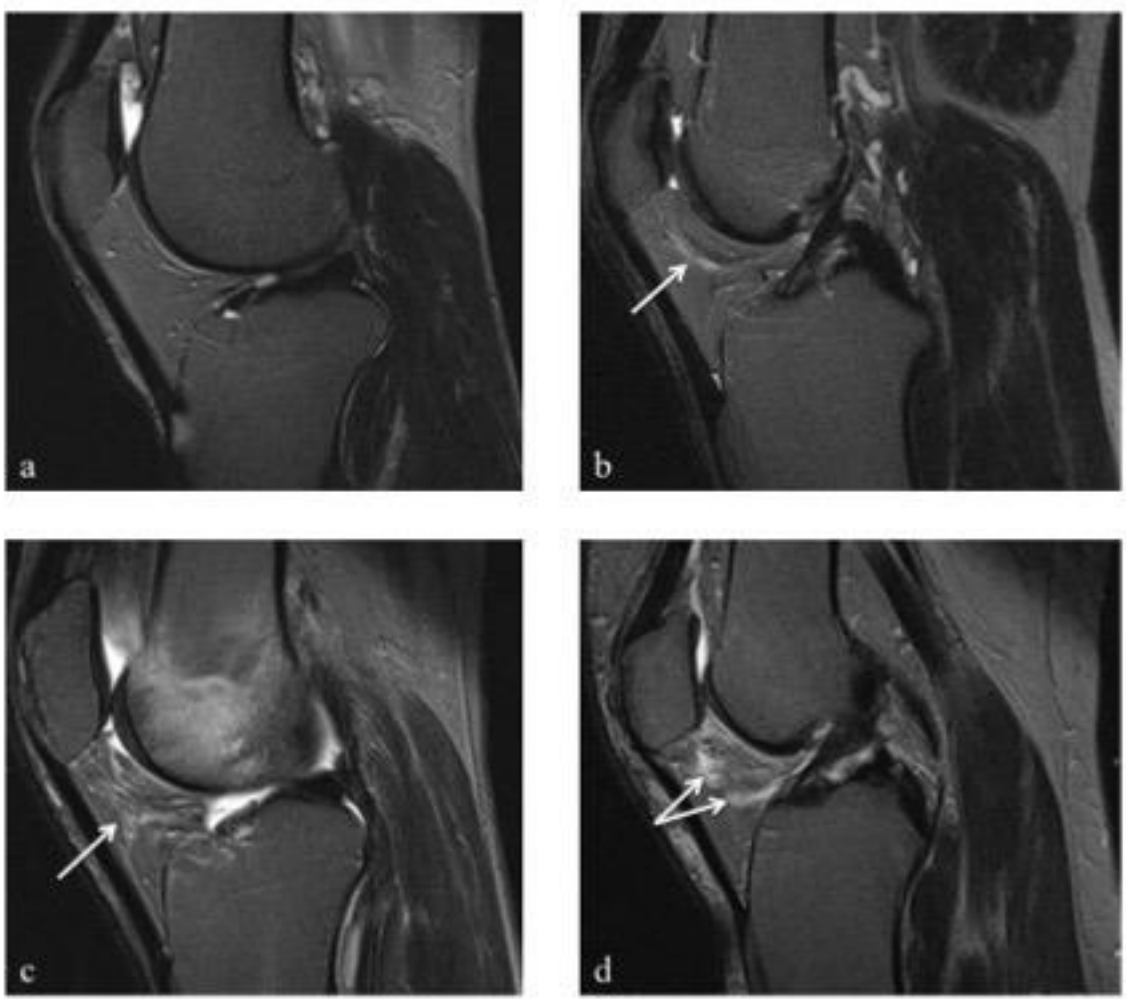

Figure 3.3 Semi-quantitative measurements of IPFP high signal intensity alteration.
A: normal IPFP; B: arrow indicates signal intensity alteration of IPFP (grade 1); C: arrow points to signal intensity alteration of IPFP (grade 2); D: arrow indicates signal intensity alteration of IPFP (grade 3).

Hypointense signals within IPFP was scored by counting imaging slices with this abnormality: grade 0 = none; grade 1 = 1- 2 slices, grade 2 = 3-5 slices, grade 3 = ≥ 6 slices. This measurement was conducted by two experienced orthopaedists trained by an experienced radiologist, and determined using T2-weighted MR images (Figure 3.4). Intraobserver and interobsever reliabilities were assessed in 100 subjects with an ICC of 0.94 and an interobserver correlation coefficient of 0.88.

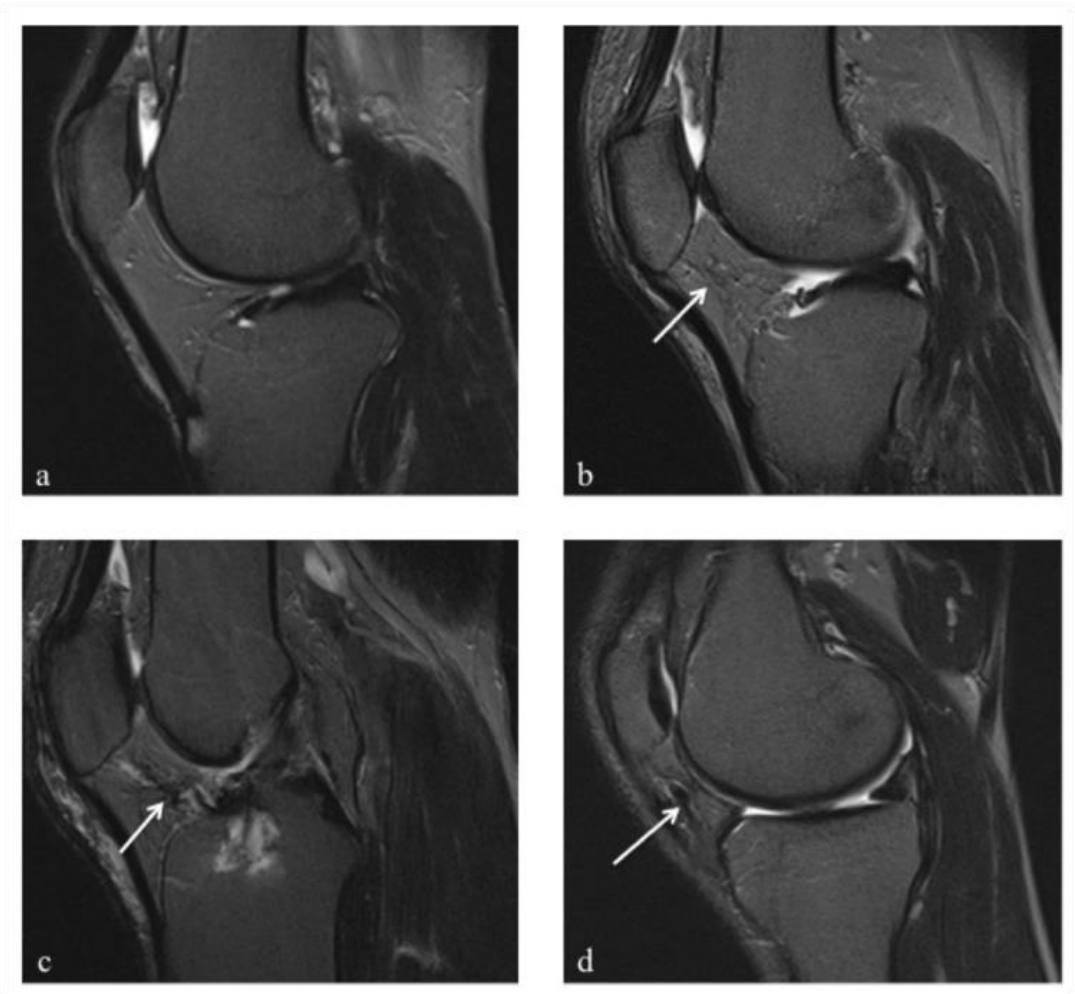

Figure 3.4 Hypointense signals on sagittal T2-weighted images with fat saturation.
A: normal IPFP; B: grade 1 hypointense signals of IPFP (arrow); C: grade 2 hypointense signals of IPFP (arrow); D: grade 3 hypointense signals of IPFP (arrow).

Quantitative measurement:

High signal intensity in IPFP was assessed (by WH) on sagittal planes of FSS T2-weighted images using MATLAB as described previously (Figure 3.5) [232]. The segmentation of IPFP was performed at ten intermediate slices in whole IPFP in order to avoiding the interference from other tissues (i.e. synovium, ligaments and subcutaneous fat) and effusion which were hard to distinguish in the beginning and ending slices. This measurement is a semi-automated procedure. An initial lasso was automatically created by a set of points selected manually near

the outer contour of IPFP and then contracted inward to approximate the real boundary of IPFP automatically. High signal intensity alterations were obtained by newly developed algorithm. Data were output automatically. We previously reported [232]: among these measurements, Clustering factor (H) and sDev (IPFP) were consistently and significantly associated with all joint structural measurements; Percentage (H) and Volume (H) were consistently and significantly associated with knee cartilage defects and ROA but not with BMLs, while Median (H) and UQ (H) were only significantly associated with tibiofemoral cartilage defects; Mean (IPFP) was not significantly associated with any knee structural measurements.

All these measures can be classified as four categories: signal intensity of whole IPFP, high signal intensity of IPFP, volume of high signal intensity and clustering effect of high signal intensity. Based on the concurrent validity and the clinical construct validity we reported, we selected one measure from each category into the current study: sDev (IPFP), UQ (H), Percentage (H) and Clustering factor (H). Of them, sDev (IPFP), being introduced to represent signal intensity variation of IPFP, is the standard deviation of whole IPFP signal intensity; UQ (H), representing the value of high signal intensity, is the upper quartile value of high signal intensity; Percentage (H), being calculated as the ratio of volume of high signal intensity region to whole IPFP volume, represents the adjusted quantity of high signal intensity; and Clustering factor (H) is calculated as:

$$\text{Clustering factor (H)} = \frac{\sum_{i=1}^{m} Vol_i / \sum_{j=1}^{n} Vol_j}{m/n}$$

where,

$$m = \begin{cases} n * 10\% & n > 10 \\ 1 & n \leq 10 \end{cases}$$

n is the number of high intensity regions. Vol_j is the volume of seed group P_j. $\sum_{j=1}^{n} Vol_j$ is the total volume of high intensity regions. $\sum_{i=1}^{m} Vol_i$ is the volume of the top m largest high intensity regions. Clustering factor (H) reflects the actual clustering effects, the bigger

$Factor_{(H)}$, the greater clustering effects. Intraobserver and interobsever reliabilities of these quantitative measurements ranged from 0.92 to 0.96 (ICC) and from 0.90 to 0.94, respectively.

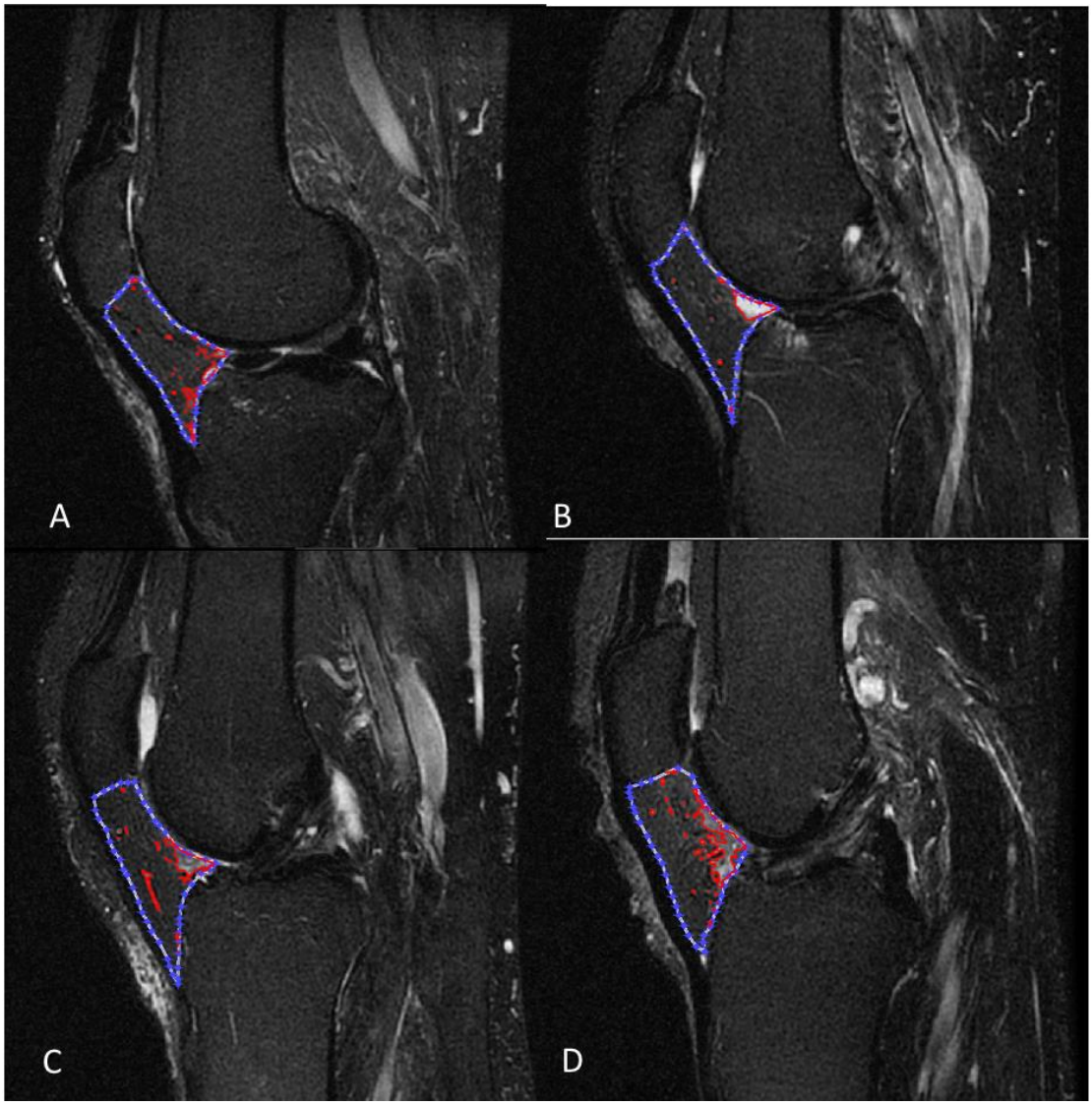

Figure 3.5 Quantitative measurements of signal intensity alteration in IPFP using MATLAB.

Semi-automatic segmentations of IPFP were performed first. Then, high signal intensity regions, showing as the red areas, were obtained by newly developed algorithm

Figure 1A has the lowest Percentage (H) and Clustering factor (H), and Figure 1B has the highest sDev (IPFP) and UQ (H). Although Figure 1B and Figure 1C have similar Percentage (H) and Clustering

factor (H), they have different sDev (IPFP) and UQ (H). Figure 1D has the biggest Percentage (H), but its sDev (IPFP) and UQ (H) are lower than Figure 1B.

3.6 Knee pain assessment

The assessment of knee pain was self-administered, using the WOMAC Index [233]. There are five categories of pain in the questionnaire (walking on flat surface, going up/down stairs, standing upright, in bed when at night, and sitting/lying). In the TASOAC study, each category of WOMAC knee pain was rated on a 10-point numeric scale from 0 (no pain) to 9 (most severe pain). In the VIDEO study, it was measured on a 100-mm visual analogue scale (score range 0–100).

3.7 Statistical analysis

T-tests and chi-squared tests were used to compare differences in means and proportions as appropriate. Standard diagnostic checks of model fit and residuals were routinely performed, and data points with large residuals and/or high influence were investigated for data errors. A p value less than 0.05 (two-tailed) is considered statistically significant. A more detailed description of statistical analyses performed is presented in their relevant chapters. All statistical analyses were performed on SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL) or Stata for Windows (version 13.0, Stata Corporation, TX, USA).

Chapter 4 Signal intensity alteration in the infrapatellar fat pad at baseline for the prediction of knee symptoms and structure in older adults: a cohort study

4.1 Introduction

OA is one of the most common diseases and is a leading cause of chronic disability in older adults [234]. It not only affects the articular cartilage and subchondral bone but also involves other structures of the joint, including menisci, synovial membrane, joint capsule, ligaments, muscles and infrapatellar fat pad [1, 64].

Obesity has been considered as the primary preventable risk factor for OA [235, 236], despite that the underlying mechanisms are not very clear. Obesity-related factors can induce pro-inflammatory cytokines and degradative enzymes which lead to cartilage matrix impairment and subchondral bone remodelling [237], suggesting that adipose tissue may act through its metabolic properties. IPFP, a local adipose tissue, has been considered as an active joint tissue in knee OA [65]. A recent review paper suggested that it would play an important role in knee OA [14]. Although the mechanisms of IPFP in pathological processes of knee OA are largely unknown, biomechanical and biochemical pathways may be involved [93, 238]. Biomechanically, IPFP may reduce the impact loading and absorb forces generated through the knee joint, and thus may play a beneficial role in knee OA. Biochemically, abnormal IPFP can produce various pro-inflammatory cytokines such as IL-1β, TNF-α, IL-6 and IL-8, , as well as various adipokines such as leptin and resistin [66-69], and thus may play a detrimental role in knee OA.

IPFP signal intensity alteration can be observed in knee OA patients using T2-or proton-density-weighted MRI [239]. Although IPFP signal intensity alteration was considered as a nonspecific feature, several clinical and epidemiological studies have utilised it as a surrogate for peripatellar synovitis [204, 205]. This type of synovitis within IPFP predicted the development of incident of radiographic OA [240]. A pathological study showed there were

vascular neoformations, fibrosis and inflammatory infiltrates in IPFP specimens obtained from patients with end-stage OA [206]; therefore, IPFP signal intensity alteration observed in MRI may be related to not only synovitis, but also other pathological changes. So far there are few studies focusing on the association between IPFP signal intensity alteration and knee symptom and structural changes so it is unclear whether IPFP signal intensity alteration is an important surrogate marker in knee OA. The aim of this study was, therefore, to determine the associations between IPFP signal intensity alteration at baseline and knee symptoms or joint structural changes in older adults with or without knee OA.

4.2 Method

4.2.1 Study design, setting and participants

This study consisted of a consecutive sample of 874 participants who had knee MRI scans at baseline from the TASOAC study, which is described in Section 3.1.

4.2.2 Assessment of knee pain

Knee pain was assessed at baseline and follow-up using the WOMAC Index, which is described in Section 3.6.

4.2.3 Knee radiographic assessment

The radiographic changes of the knee was assessed using the OARSI atlas developed by Altman et al [224], which is described in Section 3.4. The osteophytes and JSN scores were summed as the knee total ROA score, which of 1 or greater was used to define the presence of knee ROA [241].

4.2.4 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described on Section 3.5.1.

IPFP signal intensity alteration at baseline and follow-up was assessed semi-quantitatively, which is described in Section 3.5.6.

Knee cartilage volume at baseline and follow-up was assessed on T1-weighted MR images with image processing on an independent workstation, which is described in Section 3.5.2. Changes in cartilage volume were calculated as: percentage change per annum = [(follow-up volume – baseline volume)/baseline cartilage volume]/time between 2 scans in years ×100.

Knee Cartilage defects (0-4 scale) at baseline and follow-up were determined at the medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites as described in Section 3.5.3. An increase in cartilage defects was defined as a change in cartilage defects of ≥ 1.

Subchondral BMLs at baseline and follow-up were assessed on T2-weighted MR images using a semi-quantitative (0-3) scoring system as described in Section 3.5.4. An increase in BMLs was defined as a change in BMLs of ≥ 1.

Tibial plateau bone area at baseline was determined by manually measuring on axial T1-weighted MR images, as previously described [241].

4.2.5 Statistical methods

Student t or χ^2 tests were used to compare means or proportions, respectively. Multivariable linear regression analyses were used to examine the associations between IPFP signal intensity alteration (independent variable) and knee cartilage volume or change in cartilage volume (dependent variables) after adjustment for age, sex, BMI, ROA and tibial bone area. Multivariable binary logistic regression analyses were used to examine the associations between IPFP signal intensity alteration (independent variable) and knee joint space narrowing, osteophytes, as well as baseline or increases in knee cartilage defects, BMLs and knee pain (dependent variables), after adjustment for age, sex, BMI and/or ROA. Interactions between ROA status and IPFP signal intensity alteration on the outcome measures were investigated by regressing individual change in an outcome on a binary (0/1) term for ROA within IPFP signal intensity alteration, and assessed by testing the statistical significance of the coefficient of a (sex × IPFP signal intensity alteration).

4.3 Results

4.3.1 Participants

A total of 874 subjects between 50 and 80 years of age (mean, 62.1 years) took part in the present study. There were no significant differences in demographic factors (age, sex, and BMI) between these participants and those excluded (n = 226) (data not shown). Over 2.6 years, 104 subjects were lost to follow-up study due to: 25 deceased, 18 moved to other states or overseas, 12 had joint replacement, 24 physically unable, and others refused or no reason. The remaining 770 subjects completed the follow-up study; however, MRI scans were only performed on 357 of these due to decommissioning of the MRI scanner during the study. There were no significant differences in terms of age (62.5 vs 61.9 years, p=0.779), female sex (50.7% vs

49.7%, p=0.783), BMI (27.6 vs 27.8, p=0.105), and ROA (57.2% vs 60.1%, p=0.426) between these subjects and those without follow-up MRI.

Characteristics of the study population (n=874) are presented in Table 4.1. The subjects with IPFP signal intensity alteration were older, had a higher prevalence of BMLs, lateral tibiofemoral and patellar cartilage defects, and greater tibial cartilage volume and tibial bone area than those without IPFP signal intensity alteration. A greater proportion were men in those with IPFP signal intensity alteration. Additionally, subjects with IPFP signal intensity alteration had a greater proportion of radiographic OA but it was of borderline significance. There was no significant difference in BMI, patellar cartilage volume, JSN, osteophytes, WOMAC knee pain and medial tibiofemoral cartilage defects between two groups. Characteristics of the participants with complete MRI data at follow-up (n=357) are also presented in Table A.1.

4.3.2 IPFP high signal intensity alteration and cartilage defects

Cross-sectionally, IPFP signal intensity alteration was significantly and positively associated with cartilage defects in univariable analyses and these associations remained significant after adjustment for age, sex, BMI, and ROA (Table 4.2). Longitudinally, baseline IPFP signal intensity was significantly and positively associated with increases in cartilage defects at medial and lateral tibiofemoral compartments in unadjusted (Figure A.1) and multivariable analyses (Table 4.2). It was not significantly associated with an increase in patellar cartilage defects (Table 4.2).

Table 4.1 Baseline characteristics of participants based on IPFP signal intensity alteration

	IPFP signal intensity alteration No N = 443	IPFP signal intensity alteration Yes N = 431	P-value
Age (year)	**61.3 (7.3)**	**63.0 (7.2)**	**0.001**
Female sex (%)	**57.3**	**42.7**	**<0.001**
Body mass index (kg/m²)	27.5 (4.9)	28.0 (4.4)	0.119
Medial tibial cartilage volume (ml)	**2.2 (0.6)**	**2.4 (0.6)**	**0.004**
Lateral tibial cartilage volume (ml)	**2.7 (0.7)**	**2.8 (0.7)**	**0.025**
Patella cartilage volume (ml)	3.2 (0.9)	3.3 (1.0)	0.372
Medial tibial bone area (cm²)	**20.4 (3.0)**	**21.4 (3.0)**	**<0.001**
Lateral tibial bone area (cm²)	**11.8 (2.0)**	**12.5 (2.2)**	**<0.001**
Medial joint space narrowing (%)	50.4	55.3	0.158
Lateral joint space narrowing (%)	24.7	22.8	0.534
MTF osteophytes (%)	5.7	8.7	0.098
LTF osteophytes (%)	2.7	5.0	0.096
BML present (%)	**28.2**	**44.5**	**<0.001**
MTF cartilage defects (%)	21.1	26.2	0.075
LTF cartilage defects (%)	**16.4**	**26.5**	**<0.001**
Patellar cartilage defects (%)	**32.4**	**45.9**	**<0.001**
Knee pain (%)	47.1	52.2	0.128
Radiographic osteoarthritis (%)	55.6	62.3	0.052

Two-tailed t tests were used for differences between means, and χ2 tests were used for proportions (percentages). Significant differences are shown in bold. Mean (SD) except for percentages. IPFP: infrapatellar fat pat; BMI: body mass index; BML: bone marrow lesions; MTF: medial tibiofemoral; LTF: lateral tibiofemoral

4.3.3 IPFP high signal intensity alteration and BMLs

Cross-sectionally, IPFP signal intensity alteration was significantly and positively associated with BMLs in univariable and multivariable analyses (Table 4.3). Longitudinally, baseline IPFP signal intensity alteration was significantly and positively associated with increases in BMLs at all sites before (Figure A.2) and after adjustment for age, sex, BMI and ROA (Table 4.3).

Table 4.2 Associations of baseline IPFP signal intensity alteration with baseline and changes in knee cartilage defects

	Univariable OR (95% CI)	Multivariable* OR (95% CI)
Baseline cartilage defects		
Medial tibiofemoral	**1.42 (1.18, 1.71)**	**1.40 (1.13, 1.72)**
Lateral tibiofemoral	**1.67 (1.38, 2.01)**	**1.58 (1.28, 1.95)**
Patellar	**1.49 (1.25, 1.76)**	**1.56 (1.29, 1.88)**
Increase in knee cartilage defects		
Medial tibiofemoral	**1.49 (1.16, 1.93)**	**1.48 (1.12, 1.95)**
Lateral tibiofemoral	**1.61 (1.24, 2.10)**	**1.50 (1.14, 1.97)**
Patellar	1.14 (0.86, 1.51)	1.18 (0.87, 1.59)

*N=874 at baseline and n=357 at follow-up. Dependent variables: baseline cartilage defects or increases in knee cartilage defects (yes v no); independent variables: IPFP signal intensity alteration (per grade). *Adjusted for age, sex, body mass index, and radiographic osteoarthritis. IPFP: infrapatellar fat pat*

Table 4.3 Associations of baseline IPFP signal intensity alteration with baseline and changes in bone marrow lesions

	Univariable OR (95% CI)	Multivariable* OR (95% CI)
Baseline bone marrow lesions		
Any bone marrow lesions	**1.72 (1.44, 2.04)**	**1.70 (1.41, 2.05)**
Medial tibiofemoral	**1.42 (1.18, 1.71)**	**1.40 (1.14, 1.71)**
Lateral tibiofemoral	**1.82 (1.50, 2.22)**	**1.81 (1.46, 2.24)**
Patellar	**1.24 (1.00, 1.52)**	**1.30 (1.04, 1.63)**
Increases in bone marrow lesions		
Any bone marrow lesions	**1.76 (1.34, 2.32)**	**1.74 (1.30, 2.34)**
Medial tibiofemoral	**1.66 (1.21, 2.29)**	**1.56 (1.11, 2.19)**
Lateral tibiofemoral	**1.80 (1.33, 2.44)**	**1.75 (1.26, 2.42)**
Patellar	**1.45 (1.06, 2.00)**	**1.52 (1.09, 2.13)**

*N=874 at baseline and n=374 at follow-up. Dependent variables: baseline bone marrow lesions or increases in bone marrow lesions (yes v no); independent variables: IPFP signal intensity alteration (per grade). *Adjusted for age, sex, BMI, and radiographic osteoarthritis. IPFP: infrapatellar fat pat*

4.3.4 IPFP high signal intensity alteration and cartilage volume

In cross-sectional analyses, IPFP signal intensity alteration was significantly and positively associated with medial and lateral tibial cartilage volume, but not significantly associated with patellar cartilage volume. After adjustment for age, sex, BMI, radiographic OA and tibial bone area, IPFP signal intensity alteration was not significantly associated with medial and lateral cartilage volume, but was significantly and negatively associated with patellar cartilage volume (Table 4.4). The large reductions in the coefficients were largely due to adjustment for tibial bone area (data not shown). Longitudinally, baseline IPFP signal intensity alteration was not significantly associated with changes in cartilage volume at all sites in univariable analyses. After adjustment for covariates, it was negatively and significantly associated with change in lateral tibial cartilage volume, but not with changes in medial tibial and patellar cartilage volume (Table 4.4).

Table 4.4 Associations of baseline IPFP signal intensity alteration with baseline and changes in knee cartilage volume

	Univariable β (95% CI)	Multivariable* β (95% CI)
Baseline cartilage volume		
Medial tibial	**87.3 (37.0, 137.6)**	1.6 (-38.6, 42.0)
Lateral tibial	**65.6 (6.8, 124.4)**	-25.5 (-73.9, 22.9)
Patellar	13.9 (-63.9, 91.7)	**-117.1 (-186.1, -48.2)**
Change in cartilage volume		
Medial tibial	-0.3 (-1.0, 0.3)	-0.1 (-0.8, 0.6)
Lateral tibial	-0.5 (-1.0, 0.5)	**-0.5 (-1.3, -0.2)**
Patellar	-0.1 (-0.8, 0.5)	-0.1 (-0.9, 0.7)

*N=874 at baseline and n=357 at follow-up. Dependent variables: knee cartilage volume (mm^3) or change in knee cartilage volume per annum (%); independent variables: IPFP signal intensity alteration (per grade). *Adjusted for age, sex, BMI, radiographic osteoarthritis, tibial bone area. IPFP: infrapatellar fat pat*

4.3.5 IPFP high signal intensity alteration and knee pain

In cross-sectional analyses, IPFP signal intensity alteration was significantly and positively associated with prevalence of total knee pain, pain when going up/down stairs, and pain at night while in bed in univariable analyses. These associations remained, and the association with pain when sitting/lying became significant after adjustment for age, sex, BMI, and radiographic OA (Table 4.5). These associations were similar when using knee pain scores as continuous variables (date not shown). Longitudinally, baseline IPFP signal intensity alteration was only significantly associated with an increase in pain when going up/down stairs in univariable and multivariable analyses (Figure A.3 and Table 4.5).

Table 4.5 Association of baseline IPFP signal intensity alteration with baseline and increases in WOMAC knee pain

	Univariable OR (95% CI)	Multivariable* OR (95% CI)
Baseline WOMAC pain		
Total knee pain	**1.17 (1.00, 1.38)**	**1.20 (1.01, 1.44)**
Pain on flat surface	1.13 (0.94, 1.36)	1.12 (0.91, 1.37)
Pain on stairs	**1.19 (1.01, 1.40)**	**1.21 (1.01, 1.45)**
Pain in bed	**1.26 (1.05, 1.50)**	**1.34 (1.10, 1.63)**
Pain when sitting	1.19 (0.99, 1.44)	**1.23 (1.01, 1.51)**
Pain when standing	1.16 (0.96, 1.39)	1.19 (0.97, 1.48)
Increases in WOMAC pain		
Total knee pain	1.10 (0.89, 1.35)	1.05 (0.84, 1.31)
Pain on flat surface	1.09 (0.83, 1.43)	1.01 (0.82, 1.49)
Pain on stairs	**1.28 (1.03, 1.59)**	**1.29 (1.02, 1.64)**
Pain in bed	0.93 (0.71, 1.22)	0.93 (0.70, 1.24)
Pain when sitting	0.86 (0.64, 1.17)	0.90 (0.65, 1.24)
Pain when standing	1.03 (0.77, 1.37)	0.96 (0.70, 1.30)

*N=874 at baseline and n=770 at follow-up. Dependent variables: baseline WOMAC measures or increases in WOMAC measures (yes vs. no); independent variables: IPFP signal intensity alteration (per grade). *Adjusted for age, sex, BMI, and radiographic osteoarthritis. WOMAC: Western Ontario and McMasters osteoarthritis index; IPFP: infrapatellar fat pat*

4.3.6 IPFP high signal intensity alteration and radiographic OA

In cross-sectional analyses, IPFP signal intensity alteration was significantly and positively associated with ROA (OR: 1.23, P<0.05), tibiofemoral osteophytes (OR: 1.64 and 1.74, respectively, for medial and lateral compartments; both P<0.05), but not with JSN, after adjustment for age, sex and BMI.

Change in IPFP signal intensity score was positively and significantly associated only with change in cartilage defects in lateral tibiofemoral compartment (OR: 1.41, 95%CI: 1.06, 1.87), but not with changes on other structural measures (data not shown). No significant interactions between ROA status and IPFP signal intensity alteration on the outcome measures were found (data not shown), so we combined subjects with and without ROA for all analyses.

4.4 Discussion

Although there were several studies using IPFP signal intensity alteration as a surrogate for peripatellar synovitis in knee OA patients [204, 205, 240], this is a comprehensive cohort study to investigate the association of baseline IPFP signal intensity alteration with knee structure changes assessed by MRI and symptoms in older adults. We found that, cross-sectionally, IPFP signal intensity alteration was associated with increased knee symptoms, cartilage defects, BMLs, and radiographic OA, and with reduced patellar cartilage volume. Longitudinally, higher IPFP signal intensity alteration score at baseline predicted more increases in tibiofemoral cartilage defects and BMLs, greater loss of lateral tibial cartilage volume and more increases in knee symptoms. Our findings suggest that IPFP signal intensity alteration may play an important role in knee osteoarthritic changes in older adults.

Although the exact function of IPFP in knee joint is unknown, it has been considered as an active OA joint tissue [65] and may play an important role in knee OA [14]. As a component of the enthesis organ in anterior knee region [242], IPFP may help to reduce stress at attachment sites and assist in decreasing shearing forces between adjacent structures [243], and indeed, our recent study [244] reported that larger IPFP size was associated with reduced knee pain, MRI-assessed structural pathology and radiographic OA, suggesting a potentially protective

effect of IPFP size. Previous studies focusing on its biochemical components have indicated different roles of IPFP in OA processes. Bastiaansen-Jenniskens *et al* reported that fat-conditioned medium derived from IPFP of end-stage OA inhibited catabolic processes in cartilage [238]. Ushiyama et al reported that IPFP obtained from patients during knee surgery contained protein levels of basic fibroblast growth factor, vascular endothelial growth factor, TNFα, and IL-6 in homogenised tissues [104]. Klein-Wieringa et al reported that IPFP from patients with primary OA secreted higher levels of IL-6, adipsin, adiponectin and visfatin than subcutaneous adipose tissue from same patients [66]. Based on the above evidence, we conjecture that IPFP would have biphasic effects in knee OA: it may play a beneficial role physiologically (through increased size) largely due to its biomechanical or anti- catabolic properties but could also be detrimental pathologically (observed as signal intensity alteration on MRI) due to its pro-inflammatory or metabolic properties.

IPFP signal intensity alterations can be detected using T2-weighted fast spin echo MRI. Although this signal intensity alteration has been recognised as a non-specific feature in MR imaging, it was used as a surrogate for peripatellar synovitis in some clinical and epidemiological studies [204, 205], and Hoffa-synovitis has been associated with incident radiographic OA [240]. This assessment was sensitive but not specific for synovitis [245]. Pathological evidence has indicated that IPFP signal intensity alteration may represent a multitude of conditions such as chronic inflammation, vascular neoformations and edema rather than only synovitis [206]. So far, there are no direct comparisons between IPFP signal intensity alteration within whole IPFP and pathological changes in knee OA.

Knee cartilage loss is the hallmark of OA. Inflammatory and metabolic factors, such as IL-6, TNFα, basic fibroblast growth factor and leptin, have been involved in cartilage breakdown

and eventually cartilage loss [246, 247]. IPFP is the main resource of these factors, and thus could have a role to play in the degradation of articular cartilage. Our current study reported that IPFP signal intensity alteration was independently associated with focal cartilage loss observed as knee cartilage defects but inconsistently with loss of entire cartilage plates (assessed using cartilage volume). These findings suggest that IPFP signal intensity alteration may precede focal cartilage defects.

BMLs and osteophytes are the commonest subchondral bone abnormalities in knee OA. Both are associated with knee pain, cartilage defects and cartilage loss, and predict total knee replacement [178, 188, 248]. In this study, we found that IPFP signal intensity alteration was positively and consistently associated with BMLs and knee osteophytes at baseline, and also predicted increases in BMLs in all compartments over 2.6 years, suggesting that pathological changes in IPFP may affect subchondral bone abnormalities. Some biochemical and biomechanical factors would underlie this association. Leptin and VEGF have been shown to promote osteophytes formation [36, 249].

Knee pain is an important clinical outcome in knee OA. Some preliminary studies reported that signs of inflammation in IPFP (assessed using dynamic contrast-enhanced MRI) were cross-sectionally associated with knee pain in obese patients with knee OA [207], and inflammation and oedema of IPFP contributed to knee pain [207, 212]. Oedema within IPFP could increase pressure and lead to irritation of nearby tissues such as knee capsule and nociceptive nerve fibers that contain substance-P [250, 251], resulting in increased knee pain. Our current study reported that, cross-sectionally, IPFP signal intensity alteration was positively associated with knee pain (especially when going up/down stairs, at night while in bed, and when sitting or lying). Over 2.6 years, IPFP signal intensity alteration was only associated with an increase in

knee pain when going up/down stairs. Our results suggest that IPFP signal intensity alteration may play an important role in knee pain.

The main strength of this cohort study is that we selected participants randomly from the community with a large sample size and both structural and symptomatic measurements. This study has several potential limitations. First, the response rate at baseline was 57%, possibly due to the extensive protocol, which did leave the possibility open for selection bias. However, there were no significant differences in age, gender and BMI between those responded and those did not. We also had high rates of retention (with the follow-up visit completed, 88%) to offset this. Second, although the retention rate at follow-up was high, these participants only completed WOMAC questionnaires and there were 59% subjects who did not have follow-up MRIs. However, there were no significant differences in baseline characteristics between those with and without follow-up MRI. Third, radiographs were not performed at the 2.7 years follow-up, so we were unable to determine the association between IPFP quality and change in radiographic OA. Fourth, histological examinations were not able to perform in our study. Fifth, we used a change in knee pain score of ≥1 to define an increase in knee pain which may not be sensitive for our tested hypothesis; however, multiple sensitivity analyses suggested that this definition is the most appropriate. We also used pain sore ≥1 to define presence of knee pain which may not be the most clinically relevant, but the associations remained similar while using knee pain scores as continuous variables or defining prevalent knee pain using different cutpoints. Sixth, subjects with ROA (>50%) were included in the study and IPFP signal intensity could be a consequence of ROA; however, all the associations were adjusted for ROA and there were no significant interactions between ROA and IPFP signal intensity on the outcome measures, suggesting our findings were not influenced by ROA status. Last,

measurement error may influence results. However, all measures were highly reproducible suggesting this is unlikely.

In conclusion, baseline IPFP signal intensity alteration was associated with knee structural abnormalities and clinical symptoms cross-sectionally and longitudinally in older adults, suggesting that it may be an important imaging biomarker in knee OA.

Chapter 5 Hypointense signals in the infrapatellar fat pad assessed by magnetic resonance imaging are associated with knee symptoms and structure in older adults: a cohort study.

5.1 Introduction

OA is the most prevalent chronic joint disorder, characterized by pain and progressive deterioration of joint structures, and is strongly associated with risk factors such as age, female sex and obesity [252]. The most commonly affected joint is the knee, and the whole knee joint structures including articular cartilage, subchondral bone, synovium, ligaments, meniscus and periarticular fat pad can be affected in the course of knee OA [64]. Imaging biomarkers, especially from MRI, have been used in OA research for over a decade [133]. Because of its advantage in direct visualization of morphology and integrity of the whole joint, MRI has been considered as a sensitive and accurate tool to assess cartilage loss, subchondral bone abnormalities, synovitis, and ligament and meniscal lesions [138-140]. Quantitative or semi-quantitative scoring systems have been developed for evaluating these structural changes in OA [138-140].

The IPFP, the local fat around knee joint, may play an important role in the initiation and progression of knee OA [14, 65]. Biomechanically, it can promote efficient lubrication, reduce impact loading and absorb forces generated through the knee joint, which may be protective against OA [72]. Biochemically, it can produce various pro-inflammatory cytokines and adipokines, which may be deleterious to the knee joint [66-69]. Pathological examination of IPFP obtained from patients with end-stage OA found that vascular neoformations, fibrosis, and chronic inflammation were present in these specimens [206]. Dragoo et al have suggested that sagittal MRI can be used to assess abnormal IPFP quality, including fibrosis, inflammation, oedema and mass-like lesions [239].

So far, there are few clinical and epidemiological studies reporting the association between abnormal changes within IPFP and knee osteoarthritic changes. Higher signal intensity change

around IPFP assessed by T2-weighted MRI has been considered as a surrogate for peripatellar synovitis [204, 205]. Our previous study reported that high signal intensity alteration within the IPFP was associated with knee symptoms and structural changes in older adults [253]. While hyperintense signals within IPFP on T2-weighted MRI can indicate inflammation, acute haemorrhage and/or oedema, hypointense signals within IPFP on T2-weighted MRI may indicate fibrosis [239]. So far, there are no studies reporting whether hypointense signals within IPFP are associated with symptoms and structures in knee OA. The aim of this study was to describe whether hypointense signals within IPFP measured by T2-weighted MRI are associated with symptoms or joint structural abnormalities cross-sectionally and longitudinally in older adults.

5.2 Method

5.2.1 Study design, setting and participants

This study consisted of a consecutive sample of 874 participants who had knee MRI scans at baseline from the TASOAC study, which is described in Section 3.1.

5.2.2 Assessment of knee pain

Knee pain was assessed at baseline and follow-up using the WOMAC Index, which is described in Section 3.6.

5.2.3 Knee radiographic assessment

The radiographic changes of the knee was assessed using the OARSI atlas developed by Altman et al [224], which is described in Section 3.4. The osteophytes and JSN scores were summed as the knee total ROA score, which of 1 or greater was used to define the presence of knee ROA [241].

5.2.4 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described on Section 3.5.1.

IPFP hypointense signals at baseline and follow-up was assessed semi-quantitatively, which is described in Section 3.5.6.

Knee cartilage volume at baseline and follow-up was assessed on T1-weighted MR images with image processing on an independent workstation, which is described in Section 3.5.2. Changes in cartilage volume were calculated as: change per annum = (follow-up volume – baseline volume)/time between 2 scans in years.

Knee Cartilage defects (0-4 scale) at baseline and follow-up were determined at the medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites as described in Section 3.5.3. An increase in cartilage defects was defined as a change in cartilage defects of ≥ 1.

Subchondral BMLs at baseline and follow-up were assessed on T2-weighted MR images using a semi-quantitative (0-3) scoring system as described in Section 3.5.4. An increase in BMLs was defined as a change in BMLs of ≥ 1.

Tibial plateau bone area at baseline was determined by manually measuring on axial T1-weighted MR images, as previously described [241].

5.2.5 Statistical methods

Student t or χ^2 tests were used to compare means or proportions, respectively. Multivariable linear regression analyses were used to examine the associations between IPFP hypointense signals (independent variable) and knee cartilage volume or change in cartilage volume (dependent variables) after adjustment for age, sex, BMI, ROA, tibial bone area, and/or baseline cartilage volume (for change in cartilage volume) with further adjustment for cartilage defects or BMLs. Multivariable binary logistic regression analyses were used to examine the associations between IPFP hypointense signals (independent variable) and presences of knee joint space narrowing, osteophytes, as well as baseline or increases in knee cartilage defects, BMLs and WOMAC measures (dependent variables), after adjustment for covariates.

5.3 Results

5.3.1 Participants

A total of 874 subjects between 50 and 80 years of age (mean, 62.1 years) took part in the present study. There were no significant differences in demographic factors (age, sex, and BMI) between these participants and those excluded (n = 226) (data not shown). Over 2.6 years, 104 subjects were lost to follow-up study due to: 25 deceased, 18 moved to other states or overseas, 12 had joint replacement, 24 physically unable, and 25 no reason specified. The remaining 770 subjects completed the follow-up study and the first 357 had the second MRI scans but not the others as the MRI machine in the hospital was decommissioned. There were no significant differences between these subjects and those without follow-up MRI, as previously described [254].

Table 5.1 describes characteristics of the study population. There was no significant difference in age, patellar cartilage volume and knee pain between subjects with and without IPFP

hypointense signals; but the group with IPFP hypointense signals had a greater proportion of men, and higher prevalence of JSN, osteophytes, BMLs, cartilage defects, as well as higher BMI. Additionally, these subjects had greater tibial cartilage volume and tibial bone area.

5.3.2 IPFP hypointense signals and cartilage defects

IPFP hypointense signals were significantly and positively associated with baseline (data not shown) and increases in (Figure 5.1) cartilage defects at all compartments in unadjusted analyses. They were significantly and positively associated with all cartilage defects after adjustment for age, sex, BMI, and radiographic OA cross-sectionally and longitudinally (Table 5.2). These associations remained significant after further adjustment for BMLs, except that the longitudinal association at patellar site decreased in magnitude and became of borderline significance (Table 5.2).

Table 5.1 Baseline characteristics of participants split by presence of IPFP hypointense signal

	IPFP hypointense signal No (N = 305)	IPFP hypointense signal YES (N = 569)	P-value
Age (year)	61.6 (6.9)	62.4 (7.5)	0.090
Female sex (%)	**65.9**	**41.7**	**<0.001**
Body mass index (kg/m²)	**27.0 (4.5)**	**28.1 (4.7)**	**<0.001**
Medial tibial cartilage volume (ml)	**2.2 (0.6)**	**2.4 (0.6)**	**<0.001**
Lateral tibial cartilage volume (ml)	**2.6 (0.7)**	**2.8 (0.7)**	**<0.001**
Patella cartilage volume (ml)	3.2 (0.9)	3.2 (1.0)	0.235
Medial tibial bone area (cm²)	**19.9 (2.9)**	**21.4 (3.0)**	**<0.001**
Lateral tibial bone area (cm²)	**11.4 (1.9)**	**12.5 (2.2)**	**<0.001**
Medial joint space narrowing (%)	**32.9**	**63.3**	**<0.001**
Lateral joint space narrowing (%)	**15.2**	**28.2**	**<0.001**
MTF osteophytes (%)	**1.8**	**10.0**	**<0.001**
LTF osteophytes (%)	**0.7**	**5.5**	**0.001**
BML present (%)	**29.2**	**40.1**	**0.001**
MTF cartilage defects (%)	**10.2**	**30.8**	**<0.001**
LTF cartilage defects (%)	**11.3**	**26.7**	**<0.001**
Patellar cartilage defects (%)	**29.4**	**44.3**	**<0.001**
Knee pain (%)	46.2	51.4	0.144

Two-tailed t tests were used for differences between means, and χ2 tests were used for proportions (percentages). Significant differences are shown in bold.

Mean (SD) except for percentages. IPFP: infrapatellar fat pat; BMI: body mass index; BML: bone marrow lesions; MTF: medial tibiofemoral; LTF: lateral tibiofemoral

Table 5.2 Associations of IPFP hypointense signals with baseline knee cartilage defects and increases in knee cartilage defects over 2.6 years

	Multivariable* OR (95% CI)	Multivariable** OR (95% CI)
Baseline cartilage defects		
Any cartilage defects	**2.38 (1.87, 3.03)**	**2.24 (1.74, 2.87)**
Medial tibiofemoral	**2.93 (2.24, 3.83)**	**2.70 (2.05, 3.54)**
Lateral tibiofemoral	**2.62 (2.02, 3.42)**	**2.57 (1.95, 3.38)**
Patellar	**1.84 (1.47, 2.31)**	**1.93 (1.50, 2.47)**
Increase in knee cartilage defects		
Any cartilage defects	**2.27 (1.61, 3.21)**	**2.08 (1.46, 2.97)**
Medial tibiofemoral	**1.62 (1.21, 2.19)**	**1.55 (1.15, 2.09)**
Lateral tibiofemoral	**1.46 (1.09, 1.96)**	**1.39 (1.03, 1.88)**
Patellar	**1.38 (1.00, 1.90)**	1.36 (0.99, 1.88)

*Dependent variables: baseline cartilage defects or increases in knee cartilage defects (yes v no); independent variables: IPFP hypointense signals (per grade). *Adjusted for age, sex, BMI, and radiographic osteoarthritis. **Further adjustment for bone marrow lesions. IPFP: infrapatellar fat pat*

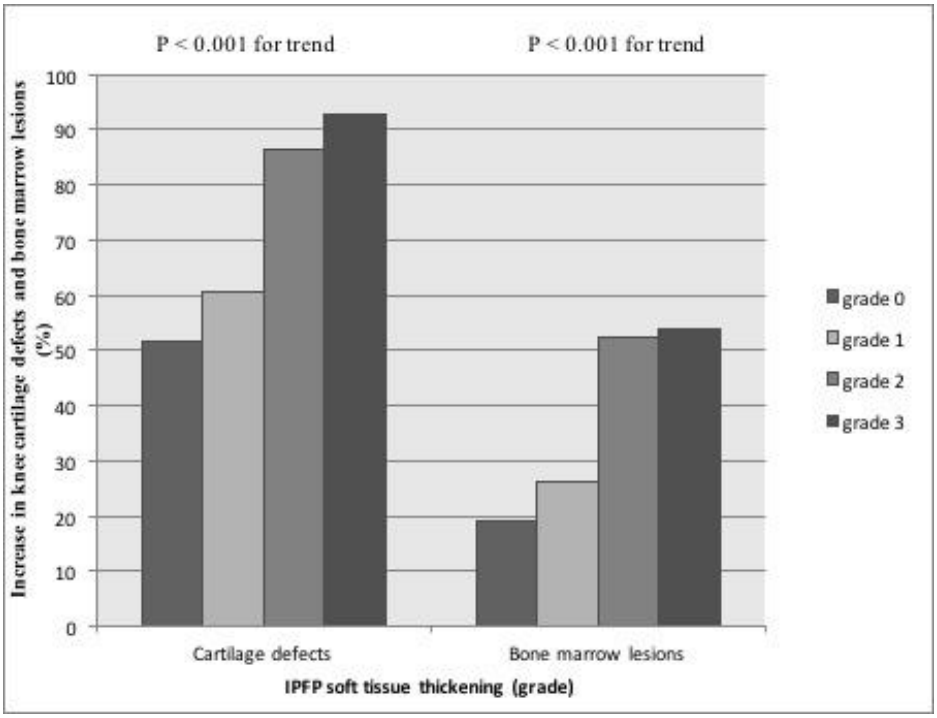

Figure 5.1 Association of IPFP hypointense signals with increase in knee cartilage defects and bone marrow lesions.

5.3.3 IPFP hypointense signals and cartilage volume

Cross-sectionally, IPFP hypointense signals were not significantly associated with medial and lateral tibial cartilage volume, but significantly and negatively associated with patellar cartilage volume after adjustment for age, sex, BMI, radiographic OA and tibial bone area (Table B.1). This significant association disappeared after further adjustment for patellar cartilage defects but remained unchanged after further adjustment for patellar BMLs (Table B.1). Longitudinally, IPFP hypointense signals were negatively and significantly associated with change in lateral tibial cartilage volume, but not with changes in medial tibial and patellar cartilage volume, after adjustment for age, sex, BMI, radiographic OA, tibial bone area and baseline cartilage volume, and this association were remained after further adjustment for cartilage defects or BMLs (Table B.1).

5.3.4 IPFP hypointense signals and BMLs

In cross-sectional analyses, IPFP hypointense signals were significantly and positively associated with any BMLs and tibiofemoral BMLs after adjustment for age, sex, BMI and radiographic OA. The associations decreased in magnitude and became non-significant after further adjustment for cartilage defects (Table 5.3). Longitudinally, IPFP hypointense signals were significantly and positively associated with increases in BMLs at all sites before (Figure 5.1) and after adjustment for age, sex, BMI and radiographic OA (Table 5.3). After further adjustment for cartilage defects, the associations decreased in magnitude and became non-significant at lateral tibiofemoral and patellar sites (Table 5.3).

Table 5.3 Associations between IPFP hypointense signals and baseline bone marrow lesions and increases in bone marrow lesions over 2.6 years

	Multivariable* OR (95% CI)	Multivariable** OR (95% CI)
Baseline bone marrow lesions		
Any bone marrow lesions	**1.64 (1.32, 2.03)**	1.11 (0.87, 1.42)
Medial tibiofemoral	**1.77 (1.39, 2.25)**	1.24 (0.95, 1.63)
Lateral tibiofemoral	**1.40 (1.08, 1.80)**	1.03 (0.78, 1.36)
Patellar	1.21 (0.93, 1.57)	0.83 (0.61, 1.13)
Increases in bone marrow lesions		
Any bone marrow lesions	**1.91 (1.39, 2.62)**	**1.45 (1.02, 2.04)**
Medial tibiofemoral	**2.11 (1.46, 3.07)**	**1.59 (1.06, 2.39)**
Lateral tibiofemoral	**1.66 (1.17, 2.35)**	1.36 (0.93, 1.97)
Patellar	**1.50 (1.04, 2.15)**	1.34 (0.92, 1.95)

*Dependent variables: baseline bone marrow lesions or increases in bone marrow lesions (yes v no); independent variables: IPFP hypointense signals (per grade). *Adjusted for age, sex, BMI, and radiographic osteoarthritis. **Further adjustment for cartilage defects. IPFP: infrapatellar fat pat*

5.3.5 IPFP hypointense signals and knee pain

IPFP hypointense signals were significantly and positively associated with total knee pain, pain when going up/down stairs, at night while in bed, and when sitting/lying after adjustment for age, sex, BMI, and radiographic OA in cross-sectional analyses, but these significant associations disappeared after further adjustment for cartilage defects or BMLs except for pain when at night while in bed (Table 5.4). Longitudinally, IPFP hypointense signals were significantly associated with an increase in total knee pain, pain when walking on flat surface, pain when going up/down stairs and when standing (Table 5.4), but these became non-significant after further adjusting for cartilage defects. The associations between IPFP hypointense signals and an increase in knee pain decreased in magnitude but became non-

significant for all knee pain subscales (except pain when going up/down stairs) after further adjustment for BMLs (Table 5.4).

Table 5.4 Association of IPFP hypointense signals with WOMAC measures and increases in WOMAC measures over 2.6 years

	Multivariable* OR (95% CI)	Multivariable** OR (95% CI)	Multivariable*** OR (95% CI)
Baseline WOMAC measures			
Total knee pain	**1.27 (1.03, 1.56)**	1.07 (0.85, 1.35)	1.15 (0.93, 1.43)
Pain on flat surface	1.22 (0.97, 1.55)	1.02 (0.79, 1.33)	1.13 (0.88, 1.44)
Pain on stairs	**1.26 (1.02, 1.55)**	1.05 (0.84, 1.32)	1.14 (0.92, 1.42)
Pain in bed	**1.47 (1.16, 1.85)**	**1.42 (1.10, 1.82)**	**1.42 (1.12, 1.79)**
Pain when sitting	**1.29 (1.02, 1.64)**	1.25 (0.96, 1.62)	1.24 (0.97, 1.58)
Pain when standing	1.22 (0.96, 1.55)	1.10 (0.85, 1.43)	1.12 (0.87, 1.43)
Increases in WOMAC measures			
Total knee pain	**1.36 (1.05, 1.76)**	1.32 (0.99, 1.74)	**1.30 (1.00, 1.69)**
Pain on flat surface	**1.52 (1.08, 2.14)**	1.22 (0.83, 1.79)	1.33 (0.94, 1.89)
Pain on stairs	**1.51 (1.14, 2.01)**	1.34 (0.98, 1.82)	**1.42 (1.06, 1.89)**
Pain in bed	1.18 (0.85, 1.63)	1.04 (0.73, 1.49)	1.14 (0.82, 1.58)
Pain when sitting	1.25 (0.88, 1.78)	1.14 (0.77, 1.67)	1.20 (0.84, 1.71)
Pain when standing	**1.44 (1.02, 2.03)**	1.22 (0.84, 1.78)	1.38 (0.98, 1.97)

*Dependent variables: baseline WOMAC measures or increases in WOMAC measures (yes vs. no); independent variables: IPFP hypointense signals (per grade). *Adjusted for age, sex, BMI, and radiographic osteoarthritis. **Further adjustment for cartilage defects. *** Further adjustment for bone marrow lesions but not for cartilage defects. WOMAC: Western Ontario and McMasters osteoarthritis index; IPFP: infrapatellar fat pat*

5.3.5 IPFP hypointense signals and radiographic OA

In cross-sectional analyses, IPFP hypointense signals were significantly and positively associated with ROA (OR: 2.91, $P < 0.001$), tibiofemoral joint space narrowing (OR: 2.72 and

1.59, respectively, for medial and lateral compartments; both $P < 0.01$), and tibiofemoral osteophytes (OR: 4.25 and 3.34, respectively, for medial and lateral compartments; both $P < 0.001$), after adjustment for age, sex and BMI.

Higher grade of IPFP hypointense signals was significantly associated with smaller IPFP maximal area after adjustment for age, gender, BMI, and total tibial bone area (Figure B.1). The associations of IPFP hypointense signals with the above outcome measures remained unchanged after further adjustment for IPFP maximal area (data not shown).

5.4 Discussion

This study is the first study to investigate the association of IPFP hypointense signals with knee structural and symptoms changes in older adults. We found that IPFP hypointense signals were cross-sectionally associated with increased knee symptoms, cartilage defects, BMLs, and radiographic OA, and with reduced patellar cartilage volume and IPFP maximal area. Longitudinally, these signal intensity changes predicted increases in cartilage defects at all sites and BMLs, loss of lateral tibial cartilage volume and increases in knee symptoms. The associations with knee cartilage defects remained significant after adjustment for BMLs, but the associations with BMLs and knee pain were weakened after adjustment for cartilage defects. This suggests that IPFP hypointense signals are associated with increased knee cartilage defects primarily and with knee BMLs and pain secondarily in older adults.

IPFP, an intracapsular but extrasynovial structure [70], is situated in the knee under the patella, between the patellar tendon, femoral condyle and tibial plateau [65], and is structurally similar to subcutaneous adipose tissue [79]. As a deformable soft tissue within anterior compartment of knee joint, it can adapt to change contours of the articular surface and is able to distribute

synovial fluid within joint cavity to reduce articular surface cushion, besides supporting the feasibility of intra-articular ligaments [72]. Composed of a fibrous scaffold with adipose tissue and synovial recesses within it, IPFP can secrete cytokines, adipokines, and lipid mediators [66-69],[131]. Therefore, IPFP may play a biphasic role in the pathologic progression of knee abnormalities.

MRI has been used to assess signal alterations within or around IPFP, and the high signal intensity alteration was mainly considered as synovial inflammation or Hoffa synovitis [204, 240]. In addition, hypointense signals closing to the synovium within or around IPFP was regarding as chronic synovitis [213, 255]. Hoffa synovitis is recognised as a key imaging biomarker for knee OA [140], and can predict the progression of knee OA [240]. Our previous study also suggested that high signal intensity alteration within IPFP was associated with knee structural and symptomatic changes in older adults [253]. As IPFP signal intensity alteration may reflect different pathological changes, more studies are required to assess the roles of IPFP signal intensity changes in knee OA.

So far, there have been no studies reporting the clinical significance of IPFP hypointense signals observed on T2-weighted fat saturation MRI images. Low signal intensity changes within IPFP may represent fibrosis or postoperative scarring [70, 73], chronic inflammation progressing from acute inflammation of the synovium due to microtrauma of this tissue [212], or synovial thickening or fibrosis [213, 214]. A study compared low signal intensity changes in plantar fat pad on MRI with histological changes and reported that these signal changes corresponded to fibrosis [215]. This fibrosis can be induced by chronic inflammation in the synovium [212], or periarticular surgeries or trauma around knees [70]. Although the roles of synovitis, surgical history and trauma in knee OA have been identified [216, 217], there has

been no evidence showing that low signal intensity within IPFP assessed by MRI is associated with symptoms and knee structure changes in the knee.

In this study, we found that in older adults, IPFP hypointense signals were consistently associated with knee cartilage defects cross-sectionally and longitudinally, independent of factors such as BMLs. They were also associated with BMLs and reduced cartilage volume cross-sectionally and longitudinally, but these associations were largely dependent of cartilage defects. Further, there were significant association between IPFP hypointense signals and knee pain, but again, these associations were largely dependent of cartilage defects rather than BMLs. These suggest that IPFP hypointense signals may induce knee structural changes and symptoms starting from cartilage defects.

The mechanisms underlying the associations between IPFP low signal intensity and knee OA measures are largely unknown. These hypointense signals within synovial membrane have been considered as synovial fibrosis and were corresponding to chronic synovitis [213, 255]. This chronic synovitis can contribute to cartilage degradations and knee pain [256]. Other pathological changes such as adipocyte necrosis or adipose fibrosis may be observed as hypointense signals within IPFP in MR imagines. Fibrosis is an abnormal tissue healing process that occurs sequentially from an inflammatory response to surgery or injury of the knee and severe fibrosis was found in 33% of IPFP biopsy specimens resected from patients with end-stage knee OA [206]. Fibrosis was also found in monoiodoacetate-induced OA models and was associated with knee pain [212]. In addition, a study focusing on the effect of strenuous running on IPFP histological changes in a rat OA model reported that fibrosis within IPFP was associated with excess physical activities and related to knee pain [257]. Fibrosis within IPFP may increase cartilage contact pressures and decrease the ability of absorbing the shocking

through the knee, and thus induce the degradation of neighbouring knee structures including cartilage and subchondral bone. Furthermore, our current study found that this abnormal signal was negatively associated with IPFP maximum area, suggesting it may decrease this absorbing ability through reducing the IPFP size.

The main strength of this cohort study lies in a large sample size with the comprehensive MRI structural measurements. There are several potential limitations. First, the response rate at baseline was 57%, possibly due to the extensive protocol, which did leave the possibility open for selection bias. However, there were no significant differences in age, gender and BMI between those responded and those did not. We also had high rates of retention (82%) to offset this. Second, we did not have radiographic OA measurements at the follow-up because X-ray is insensitive for change over this short period, so we are unable to determine the association between IPFP quality and change in radiographic OA. Third, measurement error may influence results. However, all measures were highly reproducible suggesting this is unlikely. Lastly, histological examination was not able to perform in this community-based study, so the pathological changes associated with IPFP low signal intensity are unknown.

In conclusion, hypointense signals in the IPFP were associated primarily with increased knee cartilage defects and also with BMLs and knee symptoms in cross-sectional and longitudinal analyses, suggesting the abnormality represented by this signal has a potentially important role in osteoarthritis progression.

Chapter 6 Association between quantitatively measured infrapatellar fat pad high signal intensity alteration and MRI-assessed progression of knee osteoarthritis

6.1 Introduction

OA is a common cause of chronic disability in older adults and has been considered as a multifactorial condition with risk factors including genetics, aging, female sex, injury and obesity [258, 259]. Obesity is a primary preventable risk factor which has effects partly through increasing loading in the knee joint [260]. Obese adipose tissue also has endocrine function that can produce pro-inflammatory cytokines and adipokines affecting the process of joint degradation [120, 261]. Similar to systemic adipose tissue, local adipose tissue such as IPFP has the ability of secreting pro- and anti-inflammatory cytokines and various adipokines, all of which may play roles in maintenance of cartilage and bone homeostasis in the knee joint [66-69].

IPFP is structurally similar to subcutaneous adipose tissue [79] with an abundance of adipocytes, immune cells, vessels and nerve fibres [65]. Our previous studies reported that larger IPFP size had a potentially protective effect on knee symptoms and structural changes [93, 94]. Despite its capabilities of reducing the impact loading and absorbing forces generated through the knee joint, recent evidence suggests that abnormal IPFP appears to play an important role in the initiation and progression of knee OA [14] [65]. IPFP can be an important local source of age-related inflammation and a central contributor to the degradation of neighbouring tissues within the knee [27].

Inflammation within IPFP can be assessed using non-contrast enhanced MRI [262]. High signal intensity alteration of IPFP has been regarded as a surrogate of peripatellar synovitis in epidemiological studies [204, 205]. We used semi-quantitative measurements to assess signal intensity alterations in IPFP and reported that they were significantly associated with knee symptoms and structural abnormalities in older adults [263]. There are currently few studies

that have used quantitative measurements to evaluate IPFP signal intensity alterations and to describe their relationship with knee osteoarthritic abnormalities [264]. We recently established a method to quantitatively assess IPFP signal intensity alterations and reported that this method was reproducible, and associated with knee structural changes [232]. Therefore, our current study aims to describe the cross-sectional and longitudinal associations between quantitative measures of IPFP signal intensity alterations and knee structural abnormalities in patients with symptomatic knee OA.

6.2 Method

6.2.1 Study design, setting and participants

This study consisted of a sample of 261 participants who had sagittal T2-weighted MRI scans in Tasmania from VIDEO study, which is described in Section 3.2. The sample of 152 participants who had coronal T2-weighted MRI scans in Melbourne was not included in this study. Treatment and placebo groups were combined as a cohort.

6.2.2 Knee radiographic assessment

The radiographic changes of the knee was assessed using the OARSI atlas developed by Altman et al [224], which is described in Section 3.4. We summed the osteophyte and JSN scores as total knee ROA score, as previously described [149].

6.2.3 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described on Section 3.5.1.

High signal intensity in IPFP was quantitatively assessed on sagittal planes of FSS T2-weighted images using MATLAB as described in Section 3.5.6.

Knee cartilage volume at baseline and follow-up was assessed on T1-weighted MR images with image processing on an independent workstation, which is described in Section 3.5.2. Total tibial cartilage volume was summed, and change in cartilage volume per annum was defined as (follow-up – baseline)/interval.

Knee Cartilage defects (0-4 scale) at baseline and follow-up were determined at the medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites as described in Section 3.5.3. A total score was calculated as the total of subregional scores with a maximum score of 24. Presence of tibiofemoral cartilage defects was defined as any tibial or femoral cartilage defects of >= grade 2, and an increase in cartilage defects was defined as the value from (follow-up cartilage defects – baseline cartilage defects) of >= 1.

Subchondral BMLs at baseline and follow-up were assessed on T2-weighted MR images using a semi-quantitative (0-3) scoring system as described in Section 3.5.4. Any presence of tibiofemoral BMLs was defined as a BML score of >= grade 1, and an increase in BMLs was defined as the value from (follow-up BMLs – baseline BMLs) of >= 1.

6.2.4 Statistical methods

Baseline characteristics were summarised using descriptive statistics. Univariable and multivariable linear regression analyses were used to examine the associations between baseline IPFP signal intensity alteration measures (independent variables) and baseline ROA score, and baseline and change in tibial cartilage volume (dependent variables). The

associations between baseline IPFP signal intensity alteration measures (independent variables) and the presence of or increase in tibiofemoral cartilage defects and BMLs (dependent variables) over 2 years were assessed using logistic regression analyses. The associations were adjusted for age, sex and BMI in cross-sectional analyses, and adjusted for age, sex, BMI and vitamin D treatment allocation in longitudinal analyses. Interactions between the measurements of IPFP high signal intensity alterations and sex or BMI on knee structural abnormalities were assessed by testing the statistical significance of the coefficient of a (sex or BMI × measures of IPFP high signal intensity alteration).

Multivariable linear/logistic regression was used to analyse the difference in change in tibial cartilage volume or an increase in tibiofemoral cartilage defects/BMLs over 2 years among tertiles of Clustering factor (H), in Figure 2, which visualised the association between knee structural changes and Clustering factor (H).

A *p*-value < 0.05 (2-tailed) or a 95% CI not including the null (for linear regression) or 1 (for logistic regression) point was considered as statistical significance. All statistical analyses were performed on SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL).

6.3 Results

6.3.1 Participants

Characteristics of the study population are presented in Table 6.1. A total of 261 participants (49.4% females) between 50 and 79 years of age (mean, 63.0 years) took part in the present study. There were no significant differences in demographic factors (age, sex, and BMI) between these participants and those who were excluded from this study due to no sagittal knee MRI scans available (n = 152) (data not shown). The mean baseline total score of radiographic

OA was 6.9, and the mean tibial cartilage volume was 3.7 ml. Tibiofemoral cartilage defects and any tibiofemoral BMLs were present in 53.3% and 65.3% of participants, respectively. Using quantitative measurements, the values of sDev (IPFP), UQ (H), Percentage (H) and Clustering factor (H) were presented in Table 6.1.

Table 6.1 Baseline characteristics of participants

Characteristic	Values*(n = 261)
Age (years)	63.0 (7.2)
Female sex (%)	49.4
BMI (kg/m^2)	29.7 (4.9)
ROA (grade, 0 - 30)	6.9 (5.0)
Tibial cartilage volume (ml)	3.7 (1.1)
Tibiofemoral cartilage defects present (%)	53.3
Any tibiofemoral BMLs present (%)	65.3
sDev (IPFP)	7.8 (2.2)
UQ (H)	3.8 (1.0)
Percentage (H)	6.9 (1.3)
Clustering factor (H)	6.1 (0.7)

**Mean (SD) or percentage of patients. BMI: body mass index; IPFP: infrapatellar fat pad; ROA: radiographic osteoarthritis; BMLs: bone marrow lesions; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity. Presence of tibiofemoral cartilage defects was defined as any tibial or femoral cartilage defects of >= grade 2. Any presence of tibiofemoral BMLs was defined as a BML score of >= grade 1.*

6.3.2 Cross-sectional associations

All baseline IPFP signal intensity alteration measures were significantly and positively associated with total radiographic OA score and presence of tibiofemoral cartilage defects and

BMLs, but not significantly with presence of patellar cartilage defects and BMLs, in both univariable and multivariable analyses (Table C.1-C.3). Baseline Percentage (H) and Clustering factor (H) were significantly and negatively associated with tibial cartilage volume, while sDev (IPFP) and UQ (H) were significantly and negatively associated with patellar cartilage volume, in multivariable analyses (Table C.4).

6.3.2 Longitudinal associations

Cartilage volume loss

Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were significantly associated with greater tibial cartilage loss, but not associated with patellar cartilage loss, over 2 years before and after adjustment for covariates (Table 6.2). Patients with low tertile of Clustering factor (H) had 3.3% loss of cartilage volume per annum (p.a.), while those with medium tertile had 4.6% loss of cartilage volume p.a., and with high tertile had 4.9% loss of cartilage volume p.a. (Figure 6.1a). The results were similar when medial and lateral tibial cartilage volumes were analysed individually (data not shown).

Increase in cartilage defects

Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were significantly associated with higher risks of an increase in tibiofemoral cartilage defects over 2 years in both univariable and multivariable analyses, while all baseline IPFP signal intensity alteration measures were not associated with increase in patellar cartilage defects (Table 6.3). There was a significant and positive association between tertiles of Clustering factor (H) and an increase in tibiofemoral cartilage defects (Figure 6.1b).

Table 6.2 Longitudinal associations between baseline IPFP signal intensity alteration and changes in cartilage volume

	Univariable β (95% CI)	Multivariable* β (95% CI)
Change in tibial cartilage volume		
sDev (IPFP)	**-0.34 (-0.61, -0.08)**	**-0.37 (-0.65, -0.09)**
UQ (H)	**-0.90 (-1.51, -0.29)**	**-0.93 (-1.56, -0.31)**
Percentage (H)	-0.13 (-0.54, 0.28)	-0.15 (-0.56, 0.26)
Clustering factor (H)	**-1.01 (-1.66, -0.35)**	**-1.41 (-2.25, -0.57)**
Change in patellar cartilage volume		
sDev (IPFP)	-0.10 (-0.45, 0.25)	-0.05 (-0.41, 0.32)
UQ (H)	-0.21 (-0.92, 0.51)	-0.11 (-0.85, 0.62)
Percentage (H)	-0.02 (-0.58, 0.54)	-0.04 (-0.61, 0.53)
Clustering factor (H)	-0.59 (-1.73, 0.56)	-0.47 (-1.63, 0.70)

Dependent variable: cartilage volume; Independent variable: IPFP signal intensity alteration.

**Adjusted for age, gender, BMI, tibial bone area and treatment allocation.*

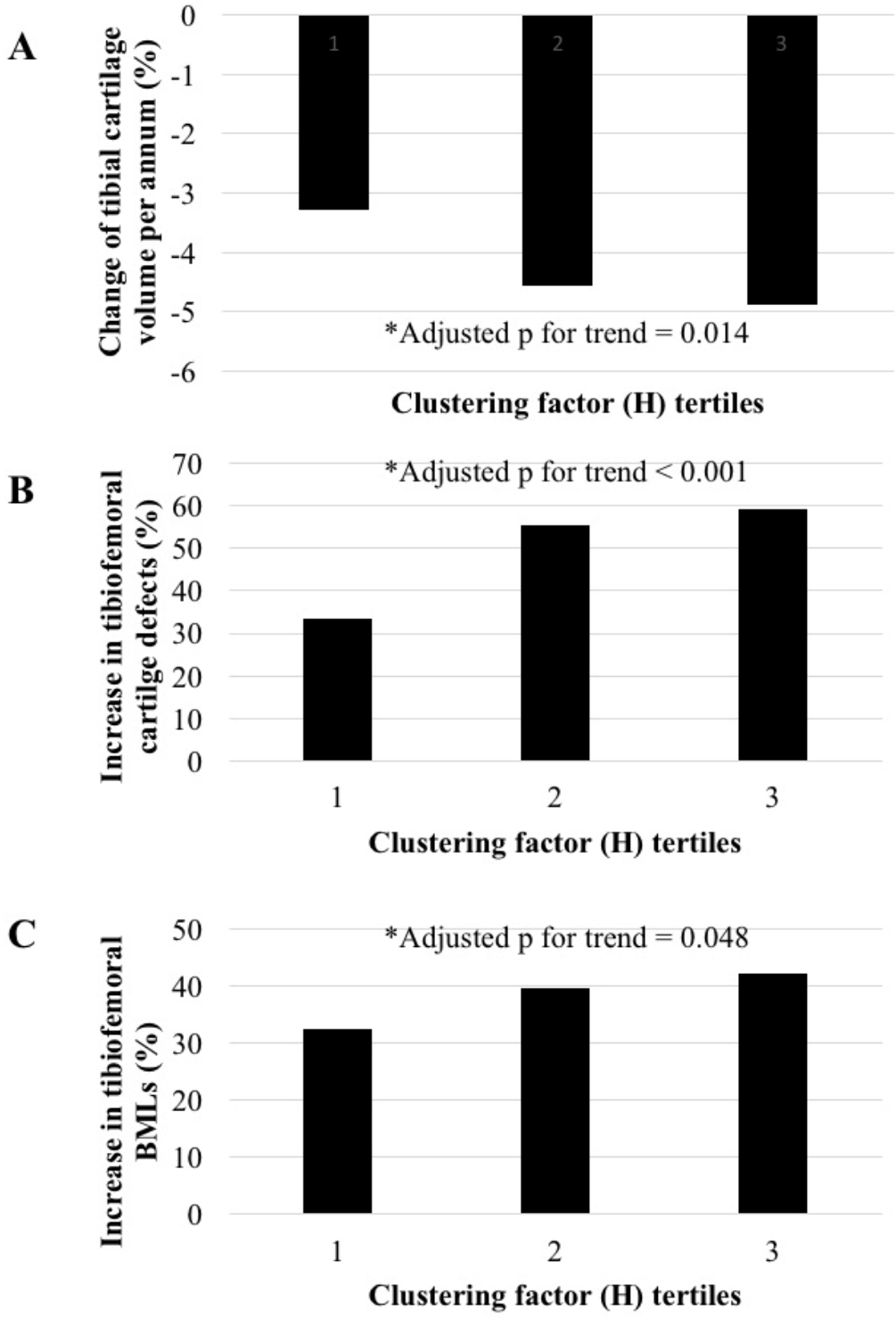

Figure 6.1 Longitudinal associations between Clustering factors of the high signal intensity alterations in IPFP and knee structural changes over 2 years.

*(A) Change of tibial cartilage volume per annum (%); (B) Increase in tibiofemoral cartilage defects (%); (C) Increase in tibiofemoral BMLs (%). *p values were those after adjustment for baseline age, gender, BMI, and treatment allocation.*

Table 6.3 Longitudinal associations between baseline IPFP signal intensity alteration and increase in cartilage defects

	Univariable RR (95% CI)	Multivariable* RR (95% CI)
Increase in tibiofemoral cartilage defects		
sDev (IPFP)	**1.33 (1.16, 1.53)**	**1.37 (1.18, 1.58)**
UQ (H)	**2.00 (1.46, 2.74)**	**2.10 (1.51, 2.92)**
Percentage (H)	0.90 (0.76, 1.08)	0.89 (0.74, 1.07)
Clustering factor (H)	**2.15 (1.44, 3.22)**	**2.19 (1.46, 3.30)**
Increase in patellar cartilage defects		
sDev (IPFP)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)
UQ (H)	0.03 (-0.01, 0.06)	0.03 (-0.01, 0.06)
Percentage (H)	-0.02 (-0.04, 0.01)	-0.03 (-0.05, 0.01)
Clustering factor (H)	0.03 (-0.02, 0.09)	0.03 (-0.02, 0.08)

Dependent variable: cartilage defects; Independent variable: IPFP signal intensity alteration.

**Adjusted for age, gender, BMI and treatment allocation.*

An increase in cartilage defects was defined as the value from (follow-up cartilage defects – baseline cartilage defects) of >= 1.

Increase in BMLs

Higher baseline Percentage (H) and Clustering factor (H) were significantly associated with higher risks of an increase in tibiofemoral BMLs over 2 years in both univariable and multivariable analyses. In contrast, there were no significant associations between all baseline IPFP signal intensity alteration measures and an increase in patellar BMLs in both analyses (Table 6.4). Tertiles of baseline Clustering factor (H) were positively associated with an increase in tibiofemoral BMLs over 2 years (Figure 6.1c).

Table 6.4 Longitudinal associations between baseline IPFP signal intensity alteration and increase in BMLs

	Univariable RR (95% CI)	Multivariable* RR (95% CI)
Increase in tibiofemoral BMLs		
sDev (IPFP)	1.09 (0.96, 1.23)	1.09 (0.96, 1.24)
UQ (H)	1.10 (0.83, 1.45)	1.08 (0.81, 1.44)
Percentage (H)	**1.28 (1.06, 1.55)**	**1.28 (1.05, 1.55)**
Clustering factor (H)	**1.47 (1.00, 2.17)**	**1.52 (1.02, 2.26)**
Increase in patellar BMLs		
sDev (IPFP)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)
UQ (H)	-0.01 (-0.04, 0.02)	-0.01 (-0.05, 0.03)
Percentage (H)	0.02 (-0.01, 0.04)	0.02 (-0.01, 0.05)
Clustering factor (H)	0.00 (-0.06, 0.06)	0.00 (-0.06, 0.06)

Dependent variable: BMLs; Independent variable: IPFP signal intensity alteration.

**Adjusted for age, gender BMI and treatment allocation.*

An increase in BMLs was defined as the value from (follow-up BMLs – baseline BMLs) of >= 1.

Baseline IPFP signal intensity alteration was positively associated with baseline effusion-synovitis but not with changes in effusion-synovitis (data not shown). There were no significant interactions between the measurements of IPFP signal intensity alterations and sex or BMI on knee structural abnormalities (data not shown); therefore, men and women, and those who were normal weight, overweight or obese were combined for analyses.

6.4 Discussion

This is the first study to describe the longitudinal associations between quantitative measures of IPFP signal intensity alteration and knee structural abnormalities in patients with symptomatic knee OA. We found that, quantitative measurements of IPFP high signal intensity alteration were significantly associated with increased ROA, tibiofemoral cartilage defects and BMLs, and reduced cartilage volume at baseline. Furthermore, most of higher baseline IPFP high signal intensity alteration measures were significantly associated with greater tibial cartilage volume loss and increases in tibiofemoral cartilage defects and BMLs over 2 years. In contrast, these quantitative measurements were not significantly associated with knee structural abnormalities at patellar site.

IPFP is the biggest adipose tissue structure within the knee. Abundant with adipocytes, immune cells and vessels [65], IPFP is an important source of inflammatory cytokines and adipokines [67-69, 120], which can be a key contributor to knee OA. A pathological study illustrated that there were vascular neoformations, fibrosis, and chronic inflammation in IPFP obtained from end-stage OA patients [206]. T2-weighted FSE MRI can be used to assess IPFP signal intensity alterations [262], and these high signal intensity alterations may represent inflammation and vascular neoformations in IPFP. Several clinical and epidemiological studies used high signal intensity alteration of IPFP as a surrogate for peripatellar synovitis [204, 205]; however, the measurement was semi-quantitative. Our study uses a quantitative assessment of IPFP signal intensity alteration, which is more objective and includes more details of IPFP signal intensity changes. In addition to the rare report of associations between IPFP signal intensity alterations and knee structural changes in patients with knee OA, the results of the current study suggested that IPFP signal intensity quantitative measurements could be considered as outcome measures in future knee OA research.

Routine radiography is considered as the gold diagnostic standard of OA in clinical practice due to the ability of revealing joint space loss and osteophytes at the joint margin, and this method provides an indirect measurement of cartilage loss [2]. The OARSI atlas developed by Altman *et al* [219] is frequently used to assess features of radiographic OA [265]. Our current study found that all measures of signal intensity alteration were positively associated with radiographic knee OA in patients with symptomatic knee OA. Our findings are consistent with two recent publications using semi-quantitative assessments of IPFP signal intensity alteration. Atukorala *et al* reported that Hoffa-synovitis (assessed as high signal intensity alterations in IPFP) strongly predicted the incidence of radiographic knee OA [201]. We recently reported signal intensity alteration score was significantly associated with radiographic knee OA in population-based older adults [263]. We were unable to evaluate the temporal relationship between IPFP signal intensity alteration and ROA, as X-ray assessment was only performed at baseline as an exclusion criteria in the original clinical trial.

Cartilage loss is generally considered as a major feature of OA progression. Inflammatory and metabolic factors, such as IL-6, TNFα and leptin, have been involved in cartilage breakdown and eventually cartilage loss[246, 247]. Klein-Wieringa *et al* reported that IPFP in patients with knee OA could secrete higher levels of inflammatory mediators (IL-6, adipsin, adiponectin, and visfatin) that contributed to the pathogenesis of cartilage loss [66]. Other studies also showed that IPFP in knee OA could release pro-inflammatory factors and adipokines that could induce cartilage degradation [67, 266]. Our previous observational study illustrated that semi-quantitative measure of IPFP signal intensity alteration was significantly and positively associated with tibiofemoral cartilage loss in older adults [263]. Consistent with these reports, we found that baseline sDev (IPFP), UQ (H) and Clustering factor (H) were

associated with tibiofemoral cartilage volume loss and cartilage defects (focal cartilage loss) in patients with symptomatic knee OA over 2 years in the current study. Both studies did not find significant associations between IPFP signal intensity alterations and patellar cartilage volume loss. In this study, nearly half of the participants were with grade 4 patellar cartilage defects, and only 15% of participants had an increase in patellar cartilage defects. Moreover, the change in patellar cartilage volume was only one third of change in tibiofemoral cartilage volume. The patellofemoral compartment had more severe disease and less changes than the tibiofemoral compartment, and this may be the reason that we did not find significant associations with changes in patellar compartment. This may also explain why we only found significantly cross-sectional associations with cartilage defects and BMLs in tibiofemoral compartment. Moreover, we found that, cross-sectionally, some measures (Percentage (H), Clustering factor (H)) were related tibial cartilage volume, while others (sDev (IPFP), UQ (H)) were significantly related to patellar cartilage volume. The reasons underlying these discrepancies are unclear but the cross-sectional findings were not the focus of our current study.

Subchondral BMLs have been considered as a hallmark of OA and been related with cartilage damage and pain in the initiation and progression of OA [188, 267]. These subchondral pathological lesions may be influenced by inflammatory mediators [268] and may have a cross-talk with IPFP pathological changes through inflammatory pathways. Our previous study reported that high signal intensity alteration score in IPFP was significantly and positively associated with BMLs in older adults, suggesting there may be a link between IPFP and subchondral bone changes in the process of knee OA [263]. Consistently, our current study reported that baseline Percentage (H) and Clustering factor (H) were associated with

tibiofemoral BMLs in patients with symptomatic knee OA, while there were no significant associations between these quantitative measures and patellar BMLs.

Our current study used a novel and a semi-automatic method to assess signal intensity alterations in IPFP with the output of quantitative measures. Using this new method, our results were consistent with previous semi-quantitative methodology, suggesting the pathology of IPFP signal intensity alteration may play an important role in the progression of knee OA. Furthermore, these quantitative measures are more objective and the results showed that Clustering factor (H), sDev (IPFP) and UQ (H), but not Percentage (H), had stronger associations with knee structure changes. These measures may reflect different magnitudes of pathological changes of IPFP, and this is why they were associated with different MRI-assessed knee structural changes of knee OA. Further pathological studies are needed to identify these differences. Overall, Clustering factor (H) was more consistently associated with all MRI-assessed knee structural changes, suggesting it may be a more useful biomarker for future research. Although this quantitative methodology focused on high rather than low signal intensity alterations in IPFP, it included more details of IPFP signal intensity alteration than previous semi-quantitative methodology and had more advantages over this semi-quantitative one. It will enrich the MRI-based whole joint assessment of knee OA, and could be included in selection of participants for future clinical trials in knee OA as by excluding those in the low tertile of increased signal intensity in the IPFP thus enriching for those with faster progression of knee OA. This measure appears to provide an additional selection method, independent of age, gender, radiological grade and other structural changes.

The main strength of this study is that we used a novel quantitative measurement to assess the signal intensity alterations in IPFP. Additionally, this study included blind readings and the

standardized methods used for data acquisition with high intra- and inter- reader reliabilities. This study has several potential limitations. First, as this study is conducted as a post-hoc analysis within a subsample of a RCT it may not generalizable to general population of knee OA and needs further confirmations in the further studies. Second, MRI coronal planes were used at Melbourne so the quantitative measures in these participants were unable to be performed; however, there were no significant differences in demographic factors between participants included and excluded from this study. Third, histological examinations were not able to perform in our study so the pathological changes of IPFP signal intensity alterations are unknown. Fourth, more severe structural abnormalities in patellofemoral compartment at baseline would result in "ceiling effects" for the changes, which could be the reason why we did not detect significantly longitudinal associations in patellofemoral compartment. Future studies are required to examine these in patients without advanced disease in this compartment. Last, measurement error may influence results. However, all measures were highly reproducible suggesting this is unlikely.

In conclusion, the quantitative measures of IPFP signal intensity alteration were significantly and positively associated with knee structural abnormalities in tibiofemoral compartment over 2 years in patients with symptomatic knee OA, suggesting that the pathology of IPFP may play an important role in knee OA progression. Additionally, these quantitative measurements of IPFP signal intensity alterations could be used as an additional entry criteria in order to enrich for 'faster progressors' in studies of in knee OA.

Chapter 7 Higher serum levels of resistin are associated with knee synovitis and structural abnormalities in patients with symptomatic knee osteoarthritis

7.1 Introduction

Obesity is a prominent risk factor for OA [269]. In addition to its contribution through increased mechanical loading of the joint, obesity may also have a metabolic link with OA as positive associations between obesity and OA have not only been observed for the knee joint but also for non-weight-bearing joints such as the hand [237, 270]. This link may involve factors originating from adipose tissue including adipokines (e.g., leptin, adiponectin and resistin) and cytokines which have been shown to play roles in cartilage degradation, synovial inflammation and bone erosions, and have a potential to predict total knee replacement [43, 107, 271].

Resistin, one of the adipokines, is a polypeptide of 105 amino acids mainly produced by white adipose tissue, with the ability to promote insulin resistance in mice [113] [272]. It has been described as an inflammatory factor associated with multiple inflammatory diseases [116, 117]. Resistin can act as an endogenous ligand of TLR-4 that triggers major inflammatory pathways and has a significant role in mediating inflammatory responses [118]. Resistin induces the secretion of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α [39, 40] which have been implicated in the initiation and progression of OA [119]. Therefore, resistin may make an important contribution to the pathogenesis of knee OA.

MRI biomarkers such as cartilage defects, BMLs, Hoffa-synovitis and effusion-synovitis have been widely used in the research of OA initiation and progression [240, 273]. Semi-quantitative measurements of cartilage defects are associated with radiographic OA and decreased cartilage volume [153, 227]. BMLs predict cartilage defect progression and cartilage volume loss [188]. We previously reported that IPFP signal intensity alteration, a surrogate for Hoffa-synovitis, was significantly associated with knee structural and pain worsening [274]. Effusion-synovitis,

a measure of synovial inflammation, has also been associated with knee structural abnormalities [194]. These MRI biomarkers such as BMLs, Hoffa-synovitis and effusion-synovitis play important roles in the initiation and progression of knee OA.

To date, few studies have explored the association between resistin and OA measures, and their findings are inconsistent. Serum resistin levels were not associated with cartilage turnover and volume loss in knee OA patients [275] but were significantly and positively associated with synovial inflammation [41]. Some cross-sectional studies reported that serum levels of resistin were significantly associated with cartilage defects, BMLs and IPFP signal intensity alteration [211, 276], but not with cartilage volume[277]. The longitudinal associations between serum resistin and knee OA measures are uncertain so far. Therefore, our current study aims to describe the longitudinal associations of serum levels of resistin with knee synovitis measures and structural abnormalities in patients with knee OA.

7.2 Method

7.2.1 Study design, setting and participants

This study randomly selected 200 participants from one site of the VIDEO study, which is described in Section 3.2. Treatment and placebo groups were combined as a cohort.

7.2.2 Serum levels of resistin and knee pain assessment

Fasting blood samples were taken in the morning. The samples were first allowed to coagulate and were then centrifuged (2200rpm/1000g, 10 minutes). Samples were stored at -80°C until analysed.

Serum levels of resistin were measured at baseline and after 24 months as paired samples using multiplex assay kit (Luminex technology; MILLIPORE, USA) according to manufacturer's protocol. Each sample was analyzed in duplicate and resistin concentrations were determined based on assay standard curve. The limits of detection are 2.2 pg/ml. The CV in our hands was 3%.

Knee pain was assessed at baseline and at month 24 using a 100-mm visual analog scale.

7.2.3 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described on Section 3.5.1.

High signal intensity in IPFP was quantitatively assessed on sagittal planes of FSS T2-weighted images using MATLAB as described in Section 3.5.6.

Knee cartilage volume at baseline and follow-up was assessed on T1-weighted MR images with image processing on an independent workstation, which is described in Section 3.5.2. Total tibial cartilage volume was summed, and change in cartilage volume per annum was defined as (follow-up – baseline)/interval.

Knee Cartilage defects (0-4 scale) at baseline and follow-up were determined at the medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites as described in Section 3.5.3. A total score was calculated as the total of subregional scores with a maximum score of 24. Presence of tibiofemoral cartilage defects was defined as any tibial or femoral cartilage

defects of >= grade 2, and an increase in cartilage defects was defined as the value from (follow-up cartilage defects – baseline cartilage defects) of >= 1.

Subchondral BMLs at baseline and follow-up were assessed on T2-weighted MR images using a semi-quantitative (0-3) scoring system as described in Section 3.5.4. Any presence of tibiofemoral BMLs was defined as a BML score of >= grade 1, and an increase in BMLs was defined as the value from (follow-up BMLs – baseline BMLs) of >= 1.

Effusion-synovitis was assessed quantitatively and semi-quantitatively as described in Section 3.5.5. Total effusion-synovitis score of the whole joint was defined as the maximum score of either subregion, ranging from 0-3. Presence of effusion-synovitis was defined as an effusion-synovitis score of ≥ 2.

7.2.4 Statistical methods

A linear mixed-effects model allows to fully analyze longitudinal data of repeated measures, including both baseline and follow-up data, as well as account for correlation between repeated measures [278]. In this study, a linear mixed-effects regression was used to analyse the longitudinal associations between serum levels of resistin (independent variable) and IPFP signal intensity alteration, effusion-synovitis volume, cartilage volume, cartilage defects and BMLs (dependent variables) before and after including intercepts of age, sex, BMI and vitamin D treatment allocation in the models. Logistic mixed-effects regressions were used to analyse the associations between serum levels of resistin (independent variable) and the presence of effusion-synovitis (dependent variables) before and after including intercepts of age, sex, BMI and vitamin D treatment allocation in the models. The correlation within the repeated measures was addressed by using individual participant ID as a random effect. Interactions between

serum levels of resistin, time interval, sex or BMI were assessed by testing the statistical significance of coefficients of (time interval × serum levels of resistin, sex × serum levels of resistin or BMI × serum levels of resistin). Student's t-test or χ^2 tests were used to compare between-group differences in baseline knee structure abnormalities among tertiles of baseline serum resistin levels. Cross-sectional associations between tertiles of serum resistin levels and knee structure abnormalities were assessed by using multivariable linear or logistic regressions (adjustment for age, sex and BMI)

A p-value of < 0.05 (2-tailed) or a 95% CI not including the null point (for linear regression) or 1 (for logistic regression) was considered as statistically significant. All statistical analyses were performed on STATA version 12.0 for Mac (StataCorp, College Station, TX, USA).

7.3 Results

7.3.1 Participants

Characteristics of the study sample are presented in Table 7.1. A total of 200 participants (46.5% females) between 50 and 79 years of age (mean, 63.1 years) took part in the present study. There were no significant differences in demographic factors (age, sex, and BMI) between these participants and those who were excluded (n = 213) (data not shown). The median serum level of resistin was 36.24 ng/ml (the interquartile range: 29.27 - 47.12 ng/ml) and all participants were with detectable levels of resistin. Participants were divided into three groups based on tertiles of resistin levels. There were no statistically significant differences between three groups in terms of age, sex and BMI as well as IPFP high signal intensity alterations, total effusion-synovitis volume, tibial cartilage volume and tibiofemoral cartilage defects, but the presence of effusion-synovitis and tibiofemoral BMLs were greater in the groups with higher resistin levels (Table 7.1).

7.3.2 Resistin and IPFP high signal intensity alteration

Cross-sectionally, higher tertiles of baseline serum resistin levels were associated with greater Percentage (H) after adjustment for age, sex and BMI (Figure 7.1a). Longitudinally, in linear mixed-effects regression analyses, higher serum levels of resistin were associated with higher Percentage (H) and Clustering factor (H) before and after including intercepts of age, sex, BMI and intervention in the models (Table 7.2). There were no statistically significant associations between serum resistin and sDve (IPFP) and UQ (H) before and after adjustment for age, sex, BMI and intervention (Table 7.2).

Table 7.1 Baseline characteristics of participants based on resistin levels (n = 200)

Characteristic	Low tertile	Medium tertile	High tertile	P values*
Age (years)	62.3 (7.6)	63.1 (7.2)	63.8 (7.3)	0.494
Female sex (%)	49.3	48.5	41.8	0.636
BMI (kg/m^2)	28.9 (4.5)	29.9 (5.8)	29.7 (4.0)	0.498
sDev (IPFP)	8.1 (2.8)	8.8 (3.4)	8.9 (2.8)	0.243
UQ (H)	4.0 (1.4)	4.3 (1.5)	4.4 (1.4)	0.296
Percentage (H)	6.6 (1.7)	6.9 (1.9)	7.1 (1.8)	0.360
Clustering factor (H)	6.2 (1.0)	6.4 (0.9)	6.5 (0.8)	0.069
Total effusion-synovitis volume (ml)	10.1 (10.1)	10.1 (10.3)	10.2 (8.1)	0.945
Effusion-synovitis presence (%)	**40.3**	**63.1**	**63.6**	**0.008**
Tibial cartilage volume (ml)	3.6 (1.1)	3.7 (0.9)	3.8 (1.1)	0.523
Tibiofemoral cartilage defects (grade)	8.6 (2.9)	8.8 (3.5)	9.7 (2.7)	0.097
Tibiofemoral BMLs (grade)	**1.8 (2.2)**	**2.8 (3.4)**	**3.2 (3.1)**	**0.019**

**Mean (SD) or percentage of patients. BMI: body mass index; IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity; UQ (H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor (H): clustering factor of high signal intensity.*

7.3.2 Resistin and effusion-synovitis

Figure 7.1b showed that higher tertiles of baseline serum resistin levels were associated with a greater presence of effusion-synovitis at baseline in an adjusted analysis. Longitudinally, higher serum resistin levels were associated with greater total effusion-synovitis volume before and after adjustment for age, sex, BMI and intervention (Table 7.2). Serum levels of resistin were also positively associated with the presence of knee effusion-synovitis before and after the adjustment (Table 7.2).

Table 7.2 Associations between serum resistin and IPFP high signal intensity and joint effusion-synovitis

	Univariable β (95% CI)	Multivariable* β (95% CI)
IPFP high signal intensity alterations		
sDev (IPFP) (unit per 100ng/ml)	0.95 (-1.19, 3.10)	1.04 (-0.97, 3.05)
UQ (H) (unit per 100ng/ml)	0.39 (-0.62, 1.40)	0.49 (-0.47, 1.45)
Percentage (H) (% per 100ng/ml)	**1.52 (0.52, 2.52)**	**1.34 (0.38, 2.30)**
Clustering Factor(H) (unit per 100ng/ml)	**0.72 (0.06, 1.38)**	**0.69 (0.07, 1.31)**
Effusion-synovitis		
Volume (ml per 100ng/ml)	**0.81 (0.04, 1.58)**	**0.77 (0.01, 1.54)**
	RR (95% CI)	RR (95% CI)
Presence	**1.06 (1.02, 1.10)**	**1.06 (1.02, 1.10)**

Independent variable: serum resistin levels

Dependent variable: IPFP high signal alterations or effusion-synovitis

**Adjustment for age, sex, BMI and treatment allocation.*

Values in bold indicate statistically significant.

β: coefficient of regression; for example: β=1.34 means that an increase in 100 ng/ml of serum resistin was associated with an increase in 1.34% of Percentage (H) in multivariable analysis.

IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity; UQ (H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering Factor (H): clustering factor of high signal intensity.

7.3.2 Resistin and knee structural changes

Longitudinally, there was no association between serum levels of resistin and tibial cartilage volume (Table 7.3). However, higher serum levels of resistin were associated with greater tibiofemoral cartilage defect and BML scores before and after adjustment for age, sex, BMI and intervention (Table 7.3). There were positively associations between tertiles of baseline serum resistin levels and baseline presence of tibiofemoral cartilage defects and BMLs (Figure 7.1c and d).

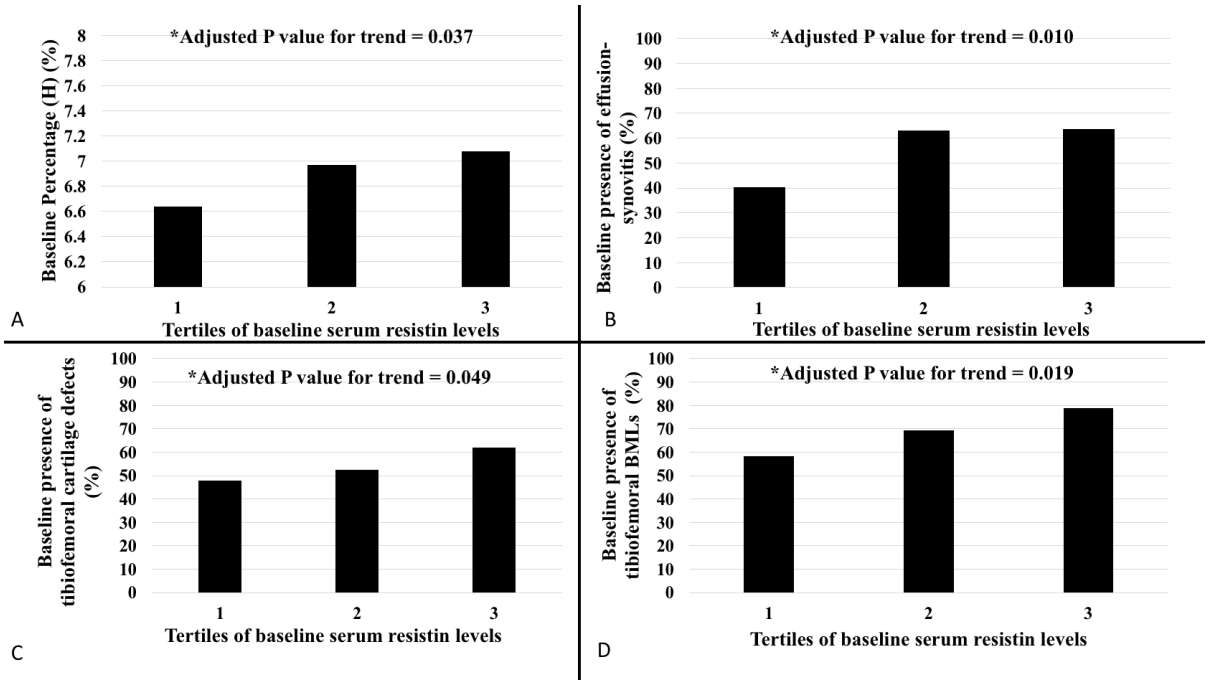

Figure 7.1 Associations between tertiles of baseline serum resistin levels and baseline knee structural abnormalities.

*(A) The percentage of IPFP high signal intensity alteration volume to whole IPFP volume [Percentage (H)]; (B) Presence of effusion-synovitis (%); (C): Presence of tibiofemoral cartilage defects (%); (D) Presence of tibiofemoral BMLs (%). *p values were those after adjustment for baseline age, sex, and BMI. Presence of effusion-synovitis was defined as an effusion-synovitis score of >= 2. Presence of tibiofemoral cartilage defects was defined as cartilage defect score at any compartment >= 2. Presence of tibiofemoral BMLs was defined as BMLs score at any compartment were of >= 1.*

Table 7.3 Association between resistin and knee structural factors

	Univariable β (95% CI)	Multivariable* β (95% CI)
Tibial cartilage volume (mm^3 per ng/ml)	-2.57 (-8.02, 2.88)	-0.92 (-6.00, 4.17)
Tibiofemoral cartilage defects (grade per 100ng/ml)	**2.22 (0.60, 3.84)**	**1.92 (0.31, 3.52)**
Tibiofemoral BMLs (grade per 100ng/ml)	**3.15 (0.99, 5.31)**	**2.97 (0.79, 5.15)**

Independent variable: serum resistin levels

Dependent variable: knee structural factors

**Adjustment for age, sex, BMI and treatment allocation.*

Values in bold indicates statistically significant.

BMLs: bone marrow lesions

The relationships between serum resistin, and IPFP signal intensity alteration, effusion-synovitis and knee structures were not modified by time interval, sex or BMI (data not shown). Serum resistin levels were not associated with cartilage volume, cartilage defects and BMLs at the patellar site (data not shown). There was no significant longitudinal association between serum resistin and knee pain (data not shown).

7.4 Discussion

In this study, Serum levels of resistin were positively associated with quantitative measures of IPFP high signal intensity alteration, effusion-synovitis volume, presence of effusion-synovitis, and scores of tibiofemoral cartilage defects and BMLs. These associations were independent of age, sex, BMI and treatment. Our results suggest that serum resistin may be linked to synovial inflammation and structural abnormalities in knee OA.

Resistin was initially investigated as an adipokine to promote insulin resistance [272]. A meta-analysis suggested that patients with OA had higher expression of resistin than healthy controls [38], but its contribution to OA pathogenesis is largely unknown. As resistin has pro-

inflammatory properties and the ability to trigger the release of pro-inflammatory cytokine such as IL-1β, IL-6 and TNF-α [39, 40], it may play an important role in OA progression. Serum levels of resistin have been shown to be correlated with increased synovial inflammation assessed histologically and positively associated with sPIIINP and the prevalence and incidence of radiographic knee OA [41, 42]. Synovial fluid levels of resistin have also been shown to be positively associated with local IL-6 levels in OA patients suggesting both systemic and local resistin may contribute to the progression of OA [43].

Although resistin has been proven to have pro-inflammatory effects that may contribute to OA pathogenesis, there are a few studies providing clinical evidence for its associations with knee OA. Several studies reported that there were cross-sectional associations between resistin and MRI assessed structural changes in knee OA [276, 277], but there are no studies describing the longitudinal associations so far. This study was the first to describe the longitudinal associations; however, it did not provide evidence for the temporal relationships between resistin and knee structural changes, which needs to be examined by further cohort studies.

Although contrast-enhanced T1-weighted MRI is the most sensitive method for quantifying knee synovitis, the need for intra-venous gadolinium adds significantly to costs and increases the risk of adverse events so it cannot be routinely used in epidemiological studies to assess knee synovitis. Instead, IPFP high signal intensity alteration assessed on unenhanced T2- or proton density-weighted fat-suppressed MRI is generally considered as a surrogate for peripatellar synovitis [204]. A pathological study showed that there were vascular neoformations, fibrosis and inflammatory infiltrates in IPFP specimens obtained from patients with end-stage OA [206]; therefore, IPFP signal intensity alteration observed in MRI may be related to not only synovitis, but also other pathological changes. Thus, we used IPFP high

signal intensity alteration in our current study. This signal alteration is related to knee pain and cartilage loss [204, 205], and predicts the incidence of radiographic knee OA [240]. We previously showed that semi-quantitative measurements of high signal intensity alterations predicted knee pain, cartilage defects, cartilage volume loss and BMLs [274]. We developed a valid and reproducible quantitative method to measure IPFP high signal intensity alteration semi-automatically [232]. In our current study, we utilised this method and found that higher serum levels of resistin were associated with higher quantitative measurements of IPFP high signal intensity alteration in patients with knee OA. These were significant for the quantity [Percentage (H)] and clustering effect [Clustering factor (H)] of IPFP high signal intensity alteration, but not for a high value [UQ (H)] and variation [sDve (IPFP)] measure of the high signal, although these associations were in a similar direction.

Effusion-synovitis is a measure of knee joint inflammation and has been shown to predict progression of OA including radiographic changes and total knee replacement [240, 273]. Effusion-synovitis is also significantly associated with knee pain and knee structural changes assessed using MRI [194, 195, 231]. In our current study, we used semi-quantitative and quantitative measurement to assess effusion-synovitis, both of which are reproducible and have clinical predictivities [194, 231]. We found significant and positive associations between serum levels of resistin and effusion-synovitis scores and volume, which is consistent with our findings for IPFP signal intensity alteration. A case-control study reported that serum levels of resistin were positively associated with histological synovial inflammation but not with cartilage degeneration in patients with knee OA [41]. Overall, these findings suggest that resistin may act as a pro-inflammatory factor in the progression of knee OA.

The hallmarks of knee OA include knee cartilage degradation and subchondral bone abnormalities. Cartilage loss, cartilage defects and BMLs assessed by MRI are generally used as imaging biomarkers in knee OA research [138, 153]. Previous studies reported that serum levels of resistin were not significantly associated with cartilage loss in patients with knee OA [275, 277]. Consistent with these studies, our current study did not find a statistically significant association between serum levels of resistin and cartilage volume. This non-significantly negative association may due to power issue or the short follow-up length. Although there have been studies to describe the associations between resistin and cartilage turnover and subchondral bone biomarkers[42, 275, 279], there are fewer studies investigating the associations between resistin and MRI-assessed cartilage defects and BMLs. Berry et al. found that serum levels of resistin had no significant associations with changes in cartilage volume and defects over 2 years [275], while another study found that serum levels of resistin was positively and significantly associated with cartilage defect and BMLs cross-sectionally [276]. Our current study found that serum levels of resistin was positively associated with cartilage defect and BML scores in patients with knee OA cross-sectionally and longitudinally.

The mechanisms underlying the associations between serum resistin and knee structures in knee OA are unknown. Previous studies have suggested that higher level of resistin were related to the expression of genes such as MMPs and aggrecanases, as well as other factors such as PGE2, PIIINP, and C-terminal propeptide of type II collagen (CTX-II) [42, 280]. An in vitro study also suggested that resistin induced a dose-dependent loss of proteoglycan in mice, and inhibited proteoglycan synthesis in human [279]. Resistin may affect knee structural changes through the above pathways. However, we did not find temporal relationships between resistin and knee structural changes over 2 years, as there were no significant interactions between serum resistin and time interval on knee structures and synovial inflammation. This

makes it difficult to determine the direction of effect. Resistin may induce synovial inflammation and then structural damage in knee OA. It is also possible that osteoarthritic joint tissues such IPFP and synovium could promote resistin production, or that increased resistin, knee structural damage, and inflammation may co-occur as part of the disease process.

The main strength of this study is that we had multiple measures of synovitis surrogates (including IPFP signal intensity alteration and effusion-synovitis) and knee osteoarthritic changes (cartilage loss, cartilage defects and BMLs) over 2 years. This study has several potential limitations. First, the original study was a RCT; therefore, results could be affected by the intervention. However, our analyses were adjusted for treatment allocation. Second, synovial fluid levels of resistin could not be measured in our current study and the association between local resistin levels and knee structural abnormalities remains unknown. Third, as inclusion and exclusion criteria were applied in the original RCT design, the generalizability to the general knee OA population needs to be confirmed.

In conclusion, higher serum levels of resistin were significantly associated with knee synovitis surrogate measures and structural abnormalities, suggesting a potential role of resistin in the pathogenesis of knee OA.

Chapter 8 Natural History of Infrapatellar Fat Pad High Signal Intensity Alteration and Factors associated with its Change

8.1 Introduction

Obesity is considered one of the most consistent risk factors for onset of knee OA, together with previous knee injury, female sex and aging [31]. The underlying mechanisms may include abnormal joint loading and inflamed adipose tissue producing adipokines and inflammatory mediators, resulting in cartilage degradation, synovial inflammation and bone erosions [32, 33]. Intra-articular adipose tissue has greater fibrosis, vascularisation, leucocyte and mast cell infiltration, and releases higher levels of IL-6, IL-8 and PGE_2 than autologous subcutaneous adipose tissue in end-stage OA patients [281], suggesting that local inflamed adipose tissue may play crucial roles in the progression of knee OA.

IPFP, the biggest intra-articular adipose tissue in the knee, is a source of cytokines, adipokines and growth factors [66, 104, 282], and has similar compositions of immune cells as synovium [283]. A recent study illustrated that locally produced leptin from IPFP had the ability to upregulate proteolytic enzymes inducing catabolic process on cartilage metabolism *in vitro* [266]. Another *in vitro* study showed that IPFP might be the trigger of cartilage degradation as well as synovial inflammation, and could have specific inflammatory phenotypes independent from systemic inflammation in obesity [284]. The interactions of IPFP with other neighbouring tissue (i.e. synovium, cartilage and bone) in the pathophysiology of knee OA indicate that this local adipose tissue may play an important role in knee OA [14].

Contrast-enhanced T1-weighted MRI is considered as the ideal way to measure peripatellar synovitis but it is costly and may have side effects with the contrast injection [204]. As a result, IPFP high signal intensity alteration assessed on unenhanced T2-weighted or proton density-weighted fat-suppressed MRI is generally accepted as a surrogate for peripatellar synovitis. IPFP high signal intensity alteration is associated with knee symptoms, cartilage loss and

subchondral bone damages [205, 263], predicts the development of ROA [201] and total knee arthroplasty [273] and is independently associated with OA progression [198]. IPFP high signal intensity alteration is also associated with serum levels of cytokines and adipokines [211].

So far, no study has examined how IPFP high signal intensity alteration changes over time, and whether it is related to OA risk factors and other structural measures of disease. Our current study, therefore, aims to describe the natural history of IPFP high signal intensity alteration and factors associated with its change over 2 years in patients with knee OA.

8.2 Methods

8.2.1 Study design, setting and participants

This study consisted of a sample of 261 participants who had sagittal T2-weighted MRI scans in Tasmania from VIDEO study, which is described in Section 3.2. The sample of 152 participants who had coronal T2-weighted MRI scans in Melbourne was not included in this study. Treatment and placebo groups were combined as a cohort.

8.2.2 Knee radiographic assessment

The radiographic changes of the knee was assessed using the OARSI atlas developed by Altman et al [224], which is described in Section 3.4. We summed the osteophyte and JSN scores as total knee ROA score, as previously described [149].

8.2.3 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described on Section 3.5.1.

High signal intensity in IPFP was quantitatively assessed on sagittal planes of FSS T2-weighted images using MATLAB as described in Section 3.5.6. In order to describe the natural history of IPFP high signal intensity alteration (i.e. an increase, stable or a decrease) (Figure 8.1) was defined based on the least significant criterion (LSC) [285] which took measurement error and the correlation between the baseline and follow-up measurements into account. A decrease in IPFP high signal intensity alteration was defined as a decrease of more than the LSC, stable was defined as change less than the LSC and an increase was defined as an increase more than the LSC.

Knee cartilage volume at baseline and follow-up was assessed on T1-weighted MR images with image processing on an independent workstation, which is described in Section 3.5.2. Total tibial cartilage volume was summed, and change in cartilage volume per annum was defined as (follow-up – baseline)/interval.

Knee Cartilage defects (0-4 scale) at baseline and follow-up were determined at the medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites as described in Section 3.5.3. A total score was calculated as the total of subregional scores with a maximum score of 24. Presence of tibiofemoral cartilage defects was defined as any tibial or femoral cartilage defects of >= grade 2, and an increase in cartilage defects was defined as the value from (follow-up cartilage defects – baseline cartilage defects) of >= 1.

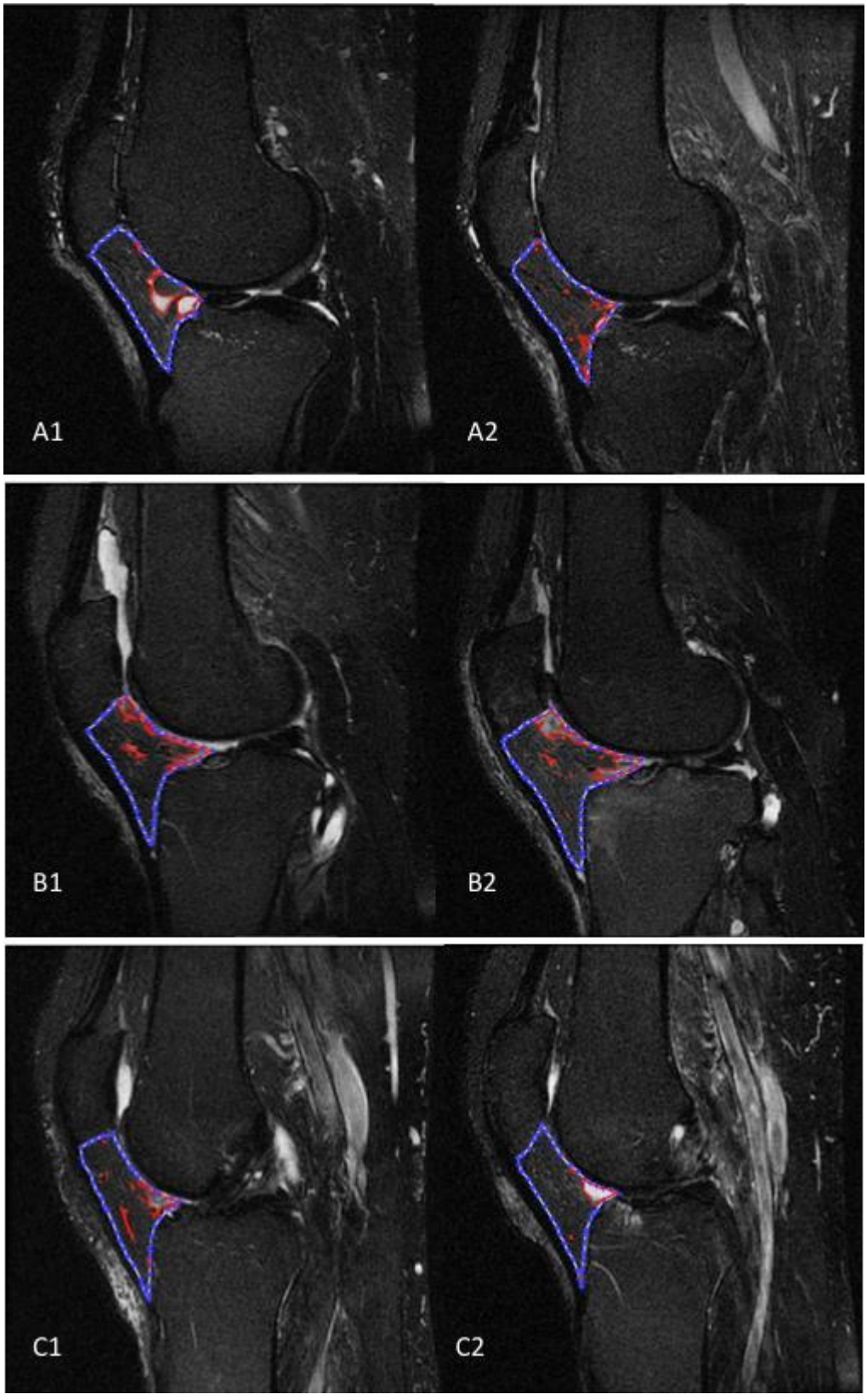

Figure 8.1. Decreases or increases of IPFP signal intensity alteration.
A1 and A2 shows the IPFP high signal intensity alteration of patient A at baseline and 2-years' follow-up, respectively. There were significant decreases in all four measures: sDev (IPFP), UQ (H),

Percentage (H) and Clustering factor(H). In contrast, patient B (B1: baseline MRI, B2: follow-up MRI) had significant increases in these four measures. Patient C, whose baseline MRI was C1 and 2-years' follow-up MRI was C2, had stable Percentage (H) and Clustering factor(H), but significant increases in sDev (IPFP) and UQ (H).

Subchondral BMLs at baseline and follow-up were assessed on T2-weighted MR images using a semi-quantitative (0-3) scoring system as described in Section 3.5.4. Any presence of tibiofemoral BMLs was defined as a BML score of >= grade 1, and an increase in BMLs was defined as the value from (follow-up BMLs – baseline BMLs) of >= 1.

Effusion-synovitis was assessed quantitatively and semi-quantitatively as described in Section 3.5.5. Total effusion-synovitis score of the whole joint was defined as the maximum score of either subregion, ranging from 0-3. Presence of effusion-synovitis was defined as an effusion-synovitis score of ≥ 2.

8.2.4 Statistical methods

Student t or χ^2 tests were used to compare means or proportions between two groups basing on any or no increase in sDev (IPFP), respectively. Multivariable linear regression analyses were used to examine the associations of demographic factors, ROA, baseline knee structures and changes in knee structures (independent variables) with changes in IPFP high signal intensity alteration (dependent variables). All analyses were adjusted for age, sex, BMI, vitamin D treatment allocation and baseline high signal intensity alteration.

A *p*-value < 0.05 (2-tailed) or a 95% CI not including the null (for linear regression) or 1 (for logistic regression) point was considered as statistically significant. All statistical analyses were performed on SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL).

8.3 Results

8.3.1 Characteristics of the study participants.

A total of 233 patients (mean 63 years, range 49-79, 42% female) were included in this study. There were no significant differences in baseline characteristics, ROA, cartilage volume or defects, BMLs and effusion-synovitis between these participants and those who were excluded from the Victoria site (data not shown). Baseline characteristics of the study sample are shown in Table 8.1. Participants were divided into two groups based on whether they had an increase in sDev (IPFP) (LSC >0.39) or not. There were no significant differences in age, female sex, BMI, total ROA scores, total cartilage volume and defects scores, BMLs and effusion-synovitis between these two groups.

Table 8.1. Baseline characteristics of participants

Characteristic	No change or decrease N = 119	Any increase* N = 104	P value
Age (years)	62.2 (6.6)	63.9 (7.9)	0.078
Female sex (%)	55.8	49.2	0.433
BMI (kg/m^2)	29.6 (4.8)	29.7 (5.0)	0.899
ROA (grade, 0 - 23)	6.5 (4.9)	7.5 (5.3)	0.173
Cartilage volume (ml)	6.1 (1.8)	5.7 (1.6)	0.068
Cartilage defects (grade, 5 – 24)	14.3 (4.2)	14.6 (4.2)	0.641
BMLs (grade, 0 – 17)	3.2 (3.0)	3.7 (3.6)	0.241
Effusion-synovitis (ml)	9.0 (8.7)	10.5 (9.8)	0.214

**Any increase was based on an increase in standard deviation of IPFP signal intensity values [sDev (IPFP)]*
Two-tailed student's t tests were used for differences between means, χ2 tests were used for proportions (percentages).
Mean (SD) or percentage of patients. BMI: body mass index; IPFP: infrapatellar fat pad; ROA: radiographic osteoarthritis; BMLs: bone marrow lesions.

8.3.2 Natural history of IPFP high signal intensity alteration.

At baseline, the mean ± SD of sDev (IPFP), UQ (H), Percentage (H) and Clustering Factor (H) were 8.4 ± 3.0 (range 3.7 – 21.5), 4.2 ± 1.4 (range 2.1 – 8.9), 6.8 ± 1.4 (range 3.6 – 9.7) and 6.3 ± 0.9 (range 4.4 – 8.9), respectively. The LSC for sDev (IPFP), UQ (H), Percentage (H) and Clustering Factor (H) were 0.39, 0.21, 0.22 and 0.12, respectively. Over 2 years, 40.4% decreased, 13% remained stable, and 46.6% increased in sDev (IPFP); 41.7% decreased, 17% remained stable, and 41.3% increased in UQ (H); 35.5% decreased, 11.3% remained stable, and 53.4% increased in Percentage (H); and 40.4% decreased, 11.2% remained stable, and 48.4% increased in Clustering Factor (H) (Figure 8.2).

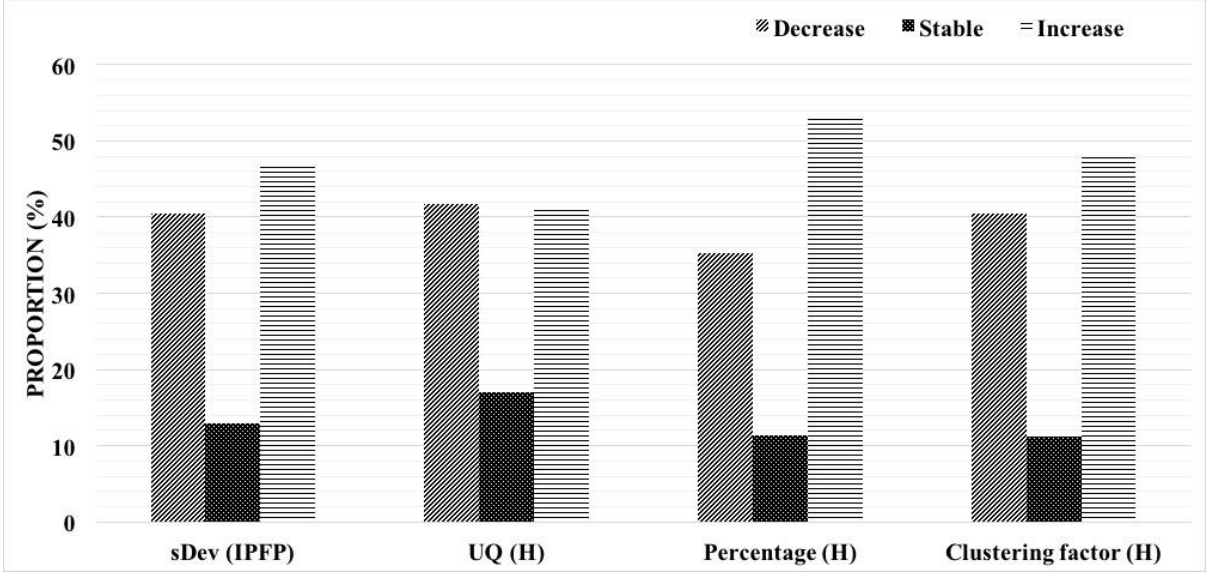

Figure 8.2. Natural history of IPFP signal intensity alteration over 2 years.
IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ (H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

Table 8.2. Associations of baseline demographic factors and ROA with changes in IPFP signal intensity alteration

	Multivariable* β (95% CI)
Changes in sDev (IPFP)	
Age (unit per year)	**0.06 (0.02, 0.11)**
Female sex (vs males)	**-0.86 (-1.53, -0.19)**
BMI (unit per kg/m^2)	0.01 (-0.07, 0.08)
ROA (unit per grade)	**0.15 (0.07, 0.22)**
Cartilage volume (unit per ml)	**-0.40 (-0.68, -0.13)**
Effusion-synovitis (unit per ml)	0.20 (-0.01, 0.40)
Changes in UQ (H)	
Age (unit per year)	**0.02 (0.01, 0.05)**
Female sex (vs males)	**-0.39 (-0.73, -0.05)**
BMI (unit per kg/m^2)	0.01 (-0.04, 0.05)
ROA (unit per grade)	**0.07 (0.03, 0.11)**
Cartilage volume (unit per ml)	**-0.17 (-0.31, -0.03)**
Effusion-synovitis (unit per ml)	**0.15 (0.04, 0.25)**

Changes in Percentage (H)	
Age (unit per year)	**0.03 (0.01, 0.05)**
Female sex (vs males)	-0.30 (-0.61, 0.02)
BMI (unit per kg/m^2)	0.01 (-0.03, 0.04)
ROA (unit per grade)	0.02 (-0.02, 0.05)
Cartilage volume (unit per ml)	-0.06 (-0.19, 0.07)
Effusion-synovitis (unit per ml)	0.01 (-0.09, 0.10)
Changes in Clustering factor (H)	
Age (unit per year)	**0.02 (0.01, 0.04)**
Female sex (vs males)	-0.09 (-0.31, 0.12)
BMI (unit per kg/m^2)	0.01 (-0.04, 0.05)
ROA (unit per grade)	**0.03 (0.01, 0.05)**
Cartilage volume (unit per ml)	-0.02 (-0.11, 0.07)
Effusion-synovitis (unit per ml)	**0.09 (0.03, 0.16)**

Dependent variable: changes in IPFP signal intensity alteration; Independent variable: baseline demographic factors or ROA.
**Adjusted for each other (for demographic factors) or age, sex and BMI (for ROA), treatment allocation and baseline IPFP signal intensity alteration.*

IPFP: infrapatellar fat pad; ROA: radiographic osteoarthritis; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

Table 8.3. Associations of changes in cartilage defects, BMLs and effusion-synovitis with changes in IPFP signal intensity alteration

	Multivariable* β (95% CI)
Changes in cartilage volume	
Changes in sDev (IPFP) (unit per ml)	-0.49 (-1.11, 0.12)
Changes in UQ (H) (unit per ml)	-0.23 (-0.54, 0.09)
Changes in Percentage (H) (unit per ml)	-0.08 (-0.37, 0.21)
Changes in Clustering factor (H) (unit per ml)	-0.06 (-0.26, 0.14)
Changes in cartilage defects	
Changes in sDev (IPFP) (unit per grade)	0.08 (-0.17, 0.28)

Changes in UQ (H) (unit per grade)	0.05 (-0.06, 0.15)
Changes in Percentage (H) (unit per grade)	**0.10 (0.01, 0.16)**
Changes in Clustering factor (H) (unit per grade)	0.01 (-0.06, 0.07)
Changes in BMLs	
Changes in sDev (IPFP) (unit per grade)	0.09 (-0.04, 0.22)
Changes in UQ (H) (unit per grade)	0.10 (-0.03, 0.24)
Changes in Percentage (H) (unit per grade)	**0.05 (0.01, 0.11)**
Changes in Clustering factor (H) (unit per grade)	**0.10 (0.04, 0.15)**
Changes in effusion-synovitis	

Changes in sDev (IPFP) (unit per ml)	**0.04 (0.01, 0.08)**
Changes in UQ (H) (unit per ml)	0.02 (-0.01, 0.04)
Changes in Percentage (H) (unit per ml)	**0.02 (0.01, 0.04)**
Changes in Clustering factor (H) (unit per ml)	**0.02 (0.01, 0.03)**

Dependent variable: changes in IPFP signal intensity alteration; Independent variable: changes in total cartilage defects, BMLs and effusion-synovitis.
**Adjusted for age, sex, BMI, treatment allocation and baseline IPFP signal intensity alteration.*
IPFP: infrapatellar fat pad; BMLs: bone marrow lesions; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

8.3.3 Associations of baseline demographic factors and knee structural abnormalities with changes in IPFP high signal intensity alteration.

Table 8.2 shows the baseline factors associated with changes in IPFP high signal intensity alteration. Age was positively associated with changes in sDev (IPFP), UQ (H), Percentage (H) and Clustering Factor (H), while female sex was negatively associated with changes in sDev (IPFP) and UQ (H), after adjustment for age or sex, BMI, treatment allocation and baseline IPFP high signal intensity alteration. There were no significant associations between BMI and changes in IPFP high signal intensity alteration in multivariable analyses.

Baseline total ROA score was positively associated with changes in sDev (IPFP), UQ (H) and Clustering Factor (H) after adjustment for covariates. Baseline total cartilage volume was negatively associated with changes in sDev (IPFP) and UQ (H) after adjustment for age, sex, BMI, treatment allocation and baseline IPFP high signal intensity alteration. There were positive associations of baseline total effusion synovitis volume with changes in UQ (H) and Clustering Factor (H) in multivariable analyses (Table 8.2).

There were no significant associations between baseline cartilage defects, BMLs and changes in IPFP signal intensity alteration in multivariable analyses (data not shown).

8.3.4 Associations between changes in joint structures and changes in IPFP high signal intensity alteration.

There was a positive association between change in cartilage defects and change in Percentage (H) after adjustment for age, sex, BMI, treatment allocation and baseline IPFP high signal intensity alteration, and change in total BMLs score was positively associated with changes in Percentage (H) and Clustering Factor (H) after adjustment for covariates (Table 8.3). Change in total effusion synovitis volume were positively associated with changes in sDev (IPFP), Percentage (H) and Clustering Factor (H) after adjustment for covariates (Table 8.3). Change in cartilage volume was not associated with changes in IPFP signal intensity alteration (Table 8.3).

8.4 Discussion

To the best of our knowledge, this study is the first to explore the natural history of IPFP high signal intensity alteration and factors associated with its change over 2 years. IPFP high signal intensity alteration was not static, with nearly 90% of signal intensity alteration measures either

worsening or improving over the study period. Changes in IPFP high signal intensity alteration were age-related and independent of BMI. Baseline higher ROA scores, less cartilage volume and more severe effusion-synovitis were associated with an increase in signal intensity alteration over 2 years. Changes in signal intensity alteration were also associated with changes in neighbouring structural factors (i.e., cartilage, subchondral bone and synovium) over time.

Our previous studies show that both semi-quantitative and quantitative measures of IPFP high signal intensity alteration were significantly and positively associated with ROA in cross-sectional analyses [232, 263]. In our present study, we found that higher grades of ROA at baseline predicted increases in IPFP high signal intensity alteration. This is consistent with another observation that patients with knee OA have greater baseline IPFP high signal intensity alteration and larger increases in these values than controls over 2 years [286]. A nested case-control studies also showed that participants with OA were more likely to experience worsening in high signal intensity alteration within IPFP over 2 years [198]. Altogether this suggest that signal intensity alteration within the IPFP is ROA-related and further studies examining the pathology of MRI-detected signal intensity alteration are needed.

Ageing is a prominent risk factor for the development and progression of OA [24]. Although the precise underlying mechanisms have not been identified, chronic low-grade inflammation may be one link between ageing and OA, as age-related low-grade systemic and local inflammation could promote OA progression [28, 287]. We found that increases in IPFP high signal intensity alteration were age-related and independent of BMI in present study. Consistently, an animal study reported that ageing was associated with significantly higher secretion of TNF-α and IL-13 from IPFP in murine [288], suggesting that age-related local adipose tissue inflammation may have an independent contribution to knee OA [27].

Interestingly, BMI was not associated with changes in IPFP high signal intensity alteration in our current study. This indicates that abnormalities in knee local adipose tissue may contribute to the process of knee OA independently of obesity. Although ageing is an unmodifiable risk factor for OA, targeting the underlying mechanism (i.e. local adipose tissue inflammation) may not be difficult to achieve.

Knee OA has been considered as a whole joint disease [64]. The cross-talk between cartilage and neighbouring tissues is important in the pathogenesis of knee OA. We found in the current study that baseline knee cartilage volume was negatively associated with increases in IPFP high signal intensity alteration, while baseline effusion-synovitis was positively associated with changes in these measures. This indicates that cartilage volume loss and effusion-synovitis could precede IPFP high signal intensity alteration in the process of knee OA. In the study, we did not find significant associations between baseline cartilage defects and BMLs with changes in signal intensity alteration. While we reported that baseline IPFP signal intensity alteration was associated with changes in cartilage defects and BMLs over 2 years in older adults [263], these suggest that IPFP high signal intensity alteration may precede cartilage defects and BMLs in disease progression of knee OA.

Further, we analysed the associations between changes in signal intensity alteration and changes in neighbouring tissues over 2 years, and found that changes of signal intensity alteration correlated to changes in effusion-synovitis, cartilage defects and BMLs. All this suggests that IPFP has potential crosstalk with neighbouring tissues such as cartilage, synovium and subchondral bone in the pathogenesis of knee OA [14]. An experimental study showed that IPFP had similar composition of immune cells from synovium [283], suggesting

that IPFP could act as the similar way as synovium in knee OA via releasing pro-inflammatory cytokines.

IPFP high signal intensity alteration measures are associated with knee symptoms, knee structural abnormalities, the progression of ROA and incidence of total knee arthroplasty [198, 201, 205, 263, 273], indicating the clinical importance of these measures in knee OA. In our current study, we found that quantitative measures of IPFP high signal intensity alteration were not static. Around 40% of participants had a decrease, and around 50% had an increase in all measures of these signal alteration. While there is no comparison data regarding the natural history of IPFP signal changes, we found that signal intensity alteration measures were more variable compared to other knee structural abnormalities [167, 178, 194]. The reason may be that this study included patients with active knee OA. We previously reported that this quantitative measurement is reproducible, and has concurrent and clinical construct validity [232]. Our finding suggests that high signal intensity alteration within IPFP may be reversible and therapeutic interventions targeting the IPFP could be considered in the future clinical practice.

This study has several potential limitations. First, as this study is conducted as a post-hoc analysis within a subsample of a RCT, the treatment allocation may influence the results. Thus, we used the multivariable analyses considering treatment allocation as a confounder. Second, MRI coronal planes were used at the Victoria site, therefore we were unable to perform our IPFP measures on these participants; however, there were no significant differences in demographic factors between participants included and excluded from this study. Last, measurement error may influence results; however, this is unlikely because all measures had

high reproducibilities, and we used LSC [285] to take measurement error and the correlation between the baseline and follow-up measurements into account.

In conclusion, IPFP high signal intensity alteration was not static in patients with knee OA. Changes in IPFP signal intensity alteration were age-related, independent of BMI, and predicted by ROA, effusion-synovitis and low cartilage volume, suggesting therapeutic interventions targeting the IPFP could be considered for OA treatment.

Chapter 9 Summary

9.1 Summary

OA is the most common joint disease in the world and it is one of the most frequent causes of pain, loss of function, and disability in Western populations [289]. It is characterized by progressive deterioration of joint structures, and mostly affects the knee joint. In Australia, it affects around 8.0% of the population, which means over 1.8 million Australians are affected by this condition [290]. It costs nearly $ 1.6 billion each year in Australia, accounted for 2.5% of total health care expenditure. Thus, it is one of the most expensive diseases. Along with the aging population and rising obesity rates, the social and economic burden associated with OA is increasing rapidly. Furthermore, there are still no proven preventative strategies and no registered effective treatments for delaying the progression of this condition. TKA is the only effective treatment for end-stage knee OA, and it is a costly procedure. Identifying modifiable risk factors and developing cost-effective treatments for this disease is urgently needed. Knee OA has been considered as a whole joint disease affecting nearly all knee structures [1, 64]. IPFP is an intracapsular but extrasynovial structure that is located in the anterior compartment of the knee [70]. It may be involved in the progression of knee OA [14]. MRI is an effective tool that can be used to assess knee structural abnormalities. This thesis has described the associations of semi-quantitative and quantitative measures of IPFP signal intensity alteration by MRI with knee pain and structural abnormalities in older populations and knee OA patients, as well as the natural history of these signal intensity alteration and factors associated with its changes. Several novel and important findings presented in this thesis are summarised below.

Chapter 4 describes the associations between semi-quantitative measures of IPFP high signal intensity alteration at baseline and knee symptoms and structural changes in older adults. In cross-sectional analyses, IPFP signal intensity alteration was significantly and positively associated with total knee pain as well as knee cartilage defects, BMLs and knee radiographic

OA and negatively associated with patellar cartilage volume after adjustment for age, sex, BMI and/or radiographic OA. Longitudinally, baseline signal intensity alteration within IPFP was significantly and positively associated with increases in knee pain when going up/down stairs as well as increases in tibiofemoral cartilage defects and BMLs, and negatively associated with change in lateral tibial cartilage volume in multivariable analyses. These results suggest that MRI assessed IPFP high signal intensity alteration may serve as an important imaging biomarker in knee OA.

While hyperintense signals within IPFP on T2-weighted MRI can indicate inflammation, acute haemorrhage and/or oedema, hypointense signals within IPFP on T2-weighted MRI may indicate fibrosis [239]. Chapter 5 describes the associations between hypointense signals in the IPFP and knee structural change and symptoms in older adults. Cross-sectionally, hypointense signals in the IPFP were significantly associated with a higher risk of knee cartilage defects at all sites, tibiofemoral BMLs and knee pain in multivariable analyses. Longitudinally, baseline signal abnormalities were significantly and positively associated with increases in knee cartilage defects, BMLs, and knee pain in multivariable analyses. The associations with cartilage defects remained significant after adjustment for BMLs, but the associations with BMLs and knee pain decreased in magnitude or became non-significant after further adjustment for cartilage defects. These results suggests the abnormality represented by this signal has a potentially important role in osteoarthritis progression.

We recently established a method to quantitatively assess IPFP signal intensity alterations and reported that this method was reproducible, and associated with knee structural changes [232]. Chapter 6 describes the cross-sectional and longitudinal associations between quantitative measures of infrapatellar fat pad (IPFP) signal intensity alteration and knee structural

abnormalities in patients with symptomatic knee OA. Higher baseline sDev (IPFP), UQ (H) and Clustering factor (H) were associated with greater loss of tibial cartilage volume and larger increases in tibiofemoral cartilage defects over 2 years. Patients with high and medium tertiles of Clustering factor (H) had greater loss of cartilage volume per annum compared with those with low tertile. Baseline Percentage (H) and Clustering factor (H) were positively and significantly associated with increases in tibiofemoral BMLs over 2 years. Cross-sectional associations between IPFP measures and knee structures were similar but more consistent. These measurements could be used as an additional entry criteria in order to enrich for 'faster progressors' in studies of knee OA.

Resistin, one of the adipokines, is a polypeptide of 105 amino acids mainly produced by white adipose tissue, with the ability to promote insulin resistance in mice [113] [272]. It has been described as an inflammatory factor associated with multiple inflammatory diseases [116, 117]. Chapter 7 describes the longitudinal associations of serum levels of resistin with IPFP signal intensity alterations and structural abnormalities in patients with knee OA. Serum resistin was positively associated with high signal intensity alteration measures of IPFP, as well as the presence and volume of effusion-synovitis in multivariable analyses. Serum levels of resistin was also positively associated with higher tibiofemoral cartilage defect and BML scores after adjustment for covariates. These results suggest the IPFP signal intensity alterations are related to resistin, which is an important adipokines. This suggests a potential link between IPFP abnormalities and knee OA.

Chapter 8 describes the natural history of IPFP high signal intensity alteration and factors associated with its change over 2 years in patients with knee OA. Over 2 years, around 40% of participants had a decrease, around 50% an increase in IPFP high signal intensity alteration,

with around 10% having no change. In multivariable analyses, baseline age, total ROA scores and effusion-synovitis volume were associated with deleterious changes in IPFP high signal intensity alteration (ie increase intensity), while baseline cartilage volume was negatively associated with changes in signal intensity alteration. Changes in cartilage defects, BMLs and effusion synovitis were associated with changes in IPFP signal intensity alteration. There was no association with BMI. IPFP high signal intensity tends to fluctuate in patients with knee OA, suggesting it may be a potential target for intervention. Further, changes in IPFP signal intensity alteration were associated with a number of demographic and structural variables but causal pathways remain uncertain.

In summary, despite the fact that pathological changes related to signal intensity alterations within IPFP are unclear, both high and low signal intensity alterations were associated with knee symptoms and structural changes cross-sectionally and longitudinally. Using semi-quantitative and quantitative measures of these signal intensity alterations, the consistent results were reached, suggesting the relevant pathological changes within IPFP may play an important role in the onset and/or progression in OA. These signal intensity alterations were associated with resistin, suggesting inflammatory adipokines may be the link between IPFP signal intensity alteration and knee osteoarthritic changes. IPFP signal intensity alterations were no static, suggesting they are treatable in knee OA patients. Future directions will be discussed in Section 9.2.

9.2 Future directions

OA has long been considered as a “wear and tear” disease leading to cartilage loss. Most recently, menisci, synovial membrane, joint capsule, ligaments, muscles and IPFP have been shown to be involved in the pathogenesis of knee OA, suggesting that knee OA is a “whole

organ" disease. IPFP, the biggest intra-articular adipose tissue in the knee, may have both mechanical and inflammatory contributions to the pathogenesis of this disease. Chapter 4 highlights that semi-quantitative measures of IPFP high signal intensity alterations are associated with knee symptoms and structural changes of knee in older adults. These measures of IPFP may reflect the pathological changes such as inflammation or vascular neoformations. These pathological changes of IPFP may reduce the "force absorbing" effects of IPFP and/or release more inflammatory cytokines or adipokines, which may be two pathways of abnormal IPFP contributing to the initiation and progression of knee OA. Although local production of inflammatory mediators are well known to cartilage degradation and synovitis, the source of inflammation are not well understood. The confirmation of low-grade inflammation in IPFP to knee OA builds the foundation for future research to examine inflammatory and metabolic mediators releasing from IPFP as important components of the disease.

Two main mechanisms, mechanical and inflammatory, are involving in the pathogenesis of knee OA. IPFP lower signal intensity alterations may reflect fibrosis or chronic inflammation, which may mechanically and biochemically contribute to the progression of knee OA. Chapter 5 has described that hypointense signals within IPFP were associated with knee symptoms and knee structural changes in older adults. The fibrosis of IPFP may decrease the capabilities of reducing the impact loading and absorbing forces generated through the knee joint, while chronic inflammatory may release more inflammatory mediators that contribute to the degradation of neighbouring tissues within the knee. However, the exact pathological changes reflected by IPFP hypointense signals are still unknow. In future research, the associations between hypointense signals of IPFP and pathological changes of IPFP could be described to fully understand the mechanism that IPFP signal intensity alteration contributes to the progression of knee OA. Furthermore, future research on the associations between releasing

inflammatory mediators from IPFP and hypointense signals of IPFP are needed to enrich the theory of local inflammation in knee OA.

IPFP has the ability of secreting pro- and anti-inflammatory cytokines and various adipokines, all of which may play roles in maintenance of cartilage and bone homeostasis in the knee joint [66-69]. Inflammation within IPFP can be assessed using non-contrast enhanced magnetic resonance imaging (MRI) [262]. The associations between semi-quantitative measures of IPFP high signal intensity alteration and knee structural changes were described in Chapter 4. There are currently few studies that have used quantitative measurements to evaluate IPFP signal intensity alterations and to describe their relationship with knee osteoarthritic abnormalities [264]. We recently established a method to quantitatively assess IPFP signal intensity alterations and reported that this method was reproducible, and associated with knee structural changes [232]. Chapter 6 illustrates these quantitative measures of IPFP signal intensity alterations were associated with MRI-assessed knee structural changes in knee OA patients. Of these measures, Clustering factor (H) was more consistently associated with all MRI-assessed knee structural changes, suggesting it may be a more useful biomarker for future research. As this study is conducted as a post-hoc analysis within a subsample of a RCT it may not generalizable to general population of knee OA and needs further confirmations in the further studies. Future studies is also needed to focus on the pathological changes related to these quantitative measures of IPFP to reveal the underlying mechanism between these abnormalities and knee OA progression.

Obesity is a prominent risk factor for OA [269]. In addition to its contribution through increased mechanical loading of the joint, obesity also has a metabolic link with OA as positive associations between obesity and OA have not only been observed for the knee joint but also

for non-weight-bearing joints such as the hand [237, 270]. This link may involve factors originating from adipose tissue including adipokines (e.g., leptin, adiponectin and resistin) and cytokines which have been shown to play roles in cartilage degradation, synovial inflammation and bone erosions, and have a potential to predict total knee replacement [43, 107, 271]. IPFP is structurally similar to subcutaneous adipose tissue and located in knee joint. Chapter 7 describes that its signal intensity alteration was associated with resistin, one of adipokines being associated with cartilage defects and BMLs [211, 276], as well as synovial inflammation [41]. In future studies, the associations between IPFP signal intensity alterations and other adipokines and inflammatory cytokines could be explored to obtain a better understanding of the underlying metabolic link between IPFP and knee OA.

Previous studies describe the significant associations of IPFP signal intensity alteration with knee symptoms, structural changes and inflammatory mediators. However, no study has examined how IPFP high signal intensity alteration changes over time, and whether it is related to OA risk factors and other structural measures of the disease. Chapter 8 describes that IPFP high signal intensity alteration was not static, with nearly 90% of signal intensity alteration measures either worsening or improving over the study period. These suggest that measures were more variable compared to other knee structural abnormalities [167, 178, 194]. The reason may be that this study included patients with active knee OA. Further research could use healthy population- based cohort studies to describe the natural history of IPFP signal intensity alteration. Changes in IPFP high signal intensity alteration were age-related and independent of BMI. Baseline higher ROA scores, less cartilage volume and more severe effusion-synovitis were associated with an increase in signal intensity alteration over 2 years. Changes in signal intensity alteration were also associated with changes in neighbouring structural factors (i.e., cartilage, subchondral bone and synovium) over time. This indicates that

abnormalities in knee local adipose tissue may contribute to the process of knee OA independently of obesity. Although ageing is an unmodifiable risk factor for OA, targeting the underlying mechanism (i.e. local adipose tissue inflammation) may not be difficult to achieve. In future, studies should examine treatments that target local adipose tissue inflammation to develop an effective therapeutic intervention.

Appendix A Appendices for Chapter 4

Table A.1 Baseline characteristics of participants based on IPFP signal intensity alteration in participants with follow-up MRI data

	IPFP signal intensity alteration No N = 136	IPFP signal intensity alteration Yes N = 221	P-value
Age (year)	62.0 (7.3)	62.8 (7.0)	0.494
Female sex (%)	**59.6**	**45.2**	**0.009**
Body mass index (kg/m²)	27.4 (4.9)	27.7 (4.1)	0.059
Medial tibial cartilage volume (ml)	2.3 (0.6)	2.4 (0.6)	0.162
Lateral tibial cartilage volume (ml)	2.7 (0.7)	2.8 (0.7)	0.619
Patella cartilage volume (ml)	3.4 (0.9)	3.3 (0.9)	0.727
Medial tibial bone area (cm²)	20.8 (3.2)	21.3 (2.9)	0.350
Lateral tibial bone area (cm²)	11.8 (2.0)	12.4 (2.1)	0.826
Medial joint space narrowing (%)	46.1	55.5	0.116
Lateral joint space narrowing (%)	19.5	19.4	1.000
MTF osteophytes (%)	3.1	8.5	0.067
LTF osteophytes (%)	0.8	3.8	0.161
BML present (%)	28.7	38.9	0.053
MTF cartilage defects (%)	**14.0**	**23.5**	**0.029**
LTF cartilage defects (%)	13.2	21.3	0.066
Patellar cartilage defects (%)	**25.7**	**43.9**	**<0.001**
Knee pain (%)	47.8	45.7	0.743
Radiographic osteoarthritis (%)	50.8	61.1	0.070

Two-tailed t tests were used for differences between means, and $\chi 2$ tests were used for proportions (percentages). Significant differences are shown in bold. Mean (SD) except for percentages. IPFP: infrapatellar fat pat; BMI: body mass index; BML: bone marrow lesions; MTF: medial tibiofemoral; LTF: lateral tibiofemoral

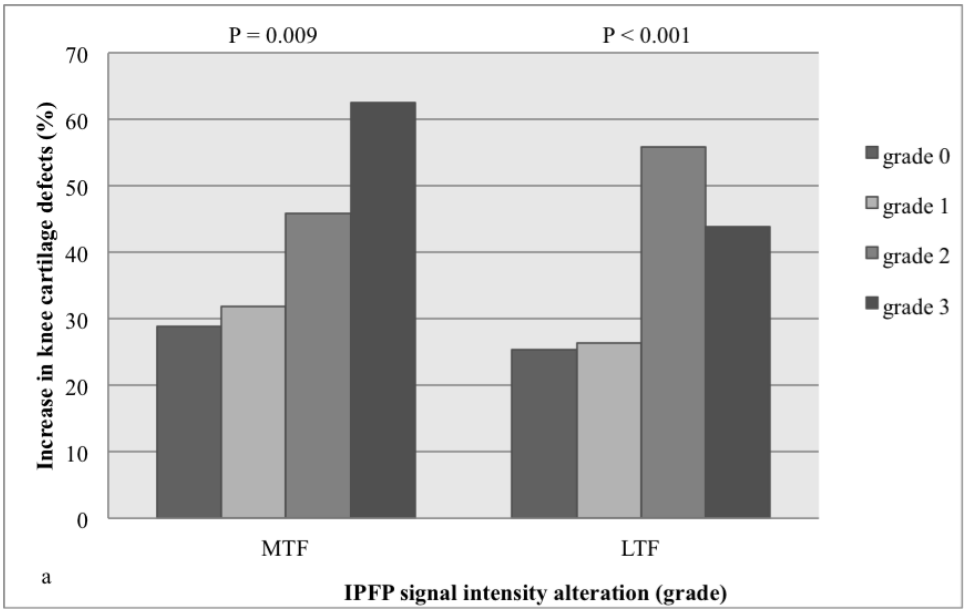

Figure A.1 Association of baseline IPFP signal intensity alteration with increases in knee cartilage defects.

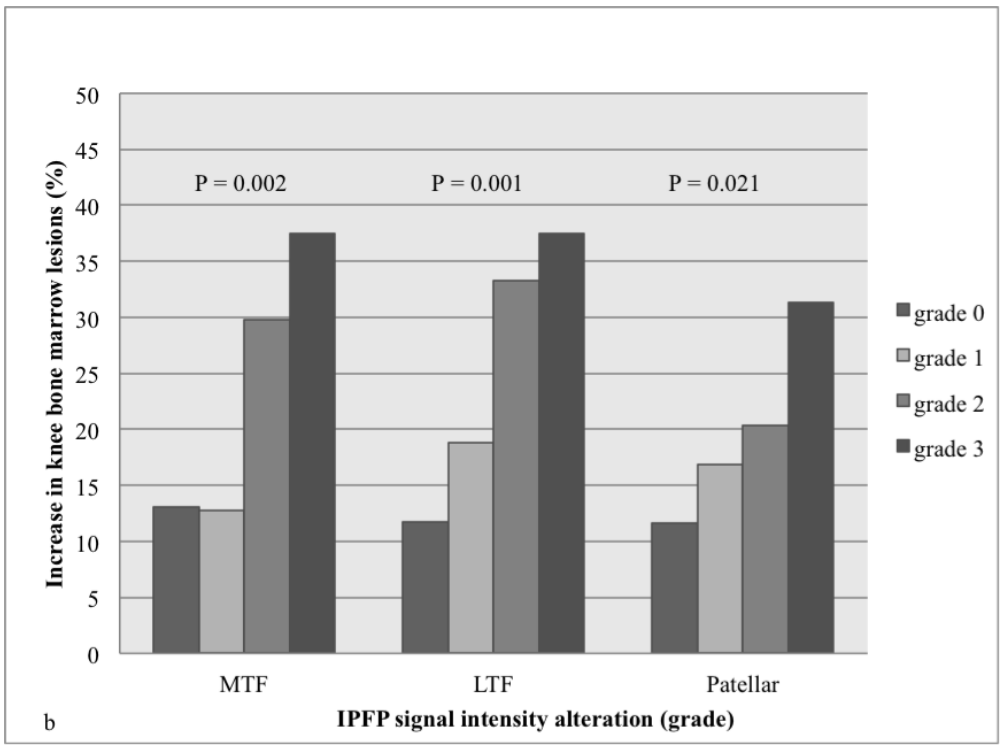

Figure A.2 Association of baseline IPFP signal intensity alteration with increases in bone marrow lesions.

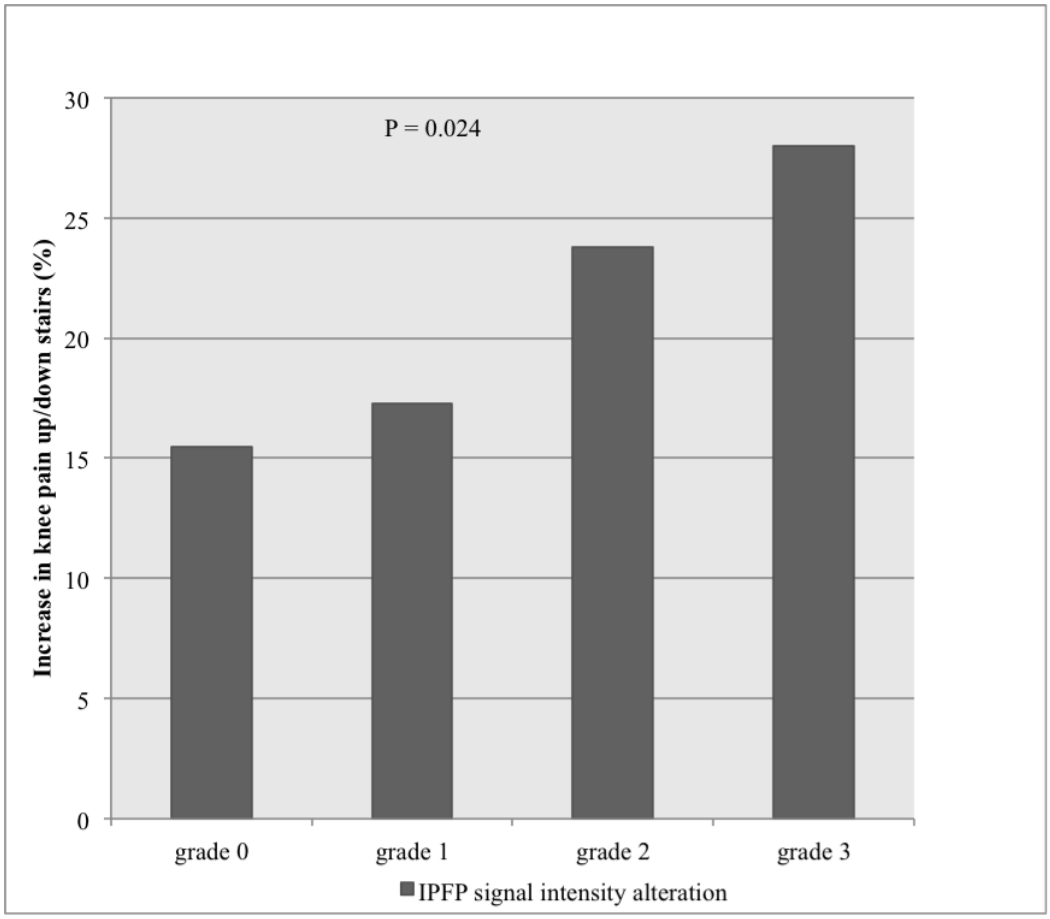

Figure A.3 Association of baseline IPFP signal intensity alteration with increase in WOMAC knee pain when going up/down stairs.

Appendix B Appendices for Chapter 5

Table B.1 Associations of IPFP hypointense signals with baseline knee cartilage volume and change in knee cartilage volume over 2.6 years

	Multivariable* β (95% CI)	Multivariable** β (95% CI)	Multivariable*** β (95% CI)
Baseline cartilage volume			
Medial tibial	-20.2 (-66.4, 26.1)	17.5 (-30.2, 65.2)	-10.3 (-57.0, 36.4)
Lateral tibial	-8.7 (-64.3, 47.0)	46.7 (-7.9, 101.2)	-5.4 (-61.2, 50.3)
Patellar	**-165.6 (-244.4, -86.7)**	-47.0 (-112.9, 18.9)	**-154.9 (-232.7, -77.2)**
Change in cartilage volume			
Medial tibial	-6.3 (-23.2, 10.6)	-3.6 (-21.1, 13.9)	-5.0 (-22.1, 12.0)
Lateral tibial	**-19.7 (-35.6, -3.8)**	**-17.0 (-33.3, -0.7)**	**- 17.8 (-33.6, -2.0)**
Patellar	-15.2 (-39.6, 9.1)	-5.7 (-29.4, 17.9)	-12.5 (-36.5, 11.5)

Dependent variables: knee cartilage volume or change in knee cartilage volume per annum (mm^3); independent variables: IPFP hypointense signals (per grade). *Adjusted for age, sex, BMI, radiographic osteoarthritis, tibial bone area, and/or baseline cartilage volume (for change in cartilage volume). **Further adjustment for cartilage defects. *** Further adjustment for bone marrow lesions but not for cartilage defects. IPFP: infrapatellar fat pat

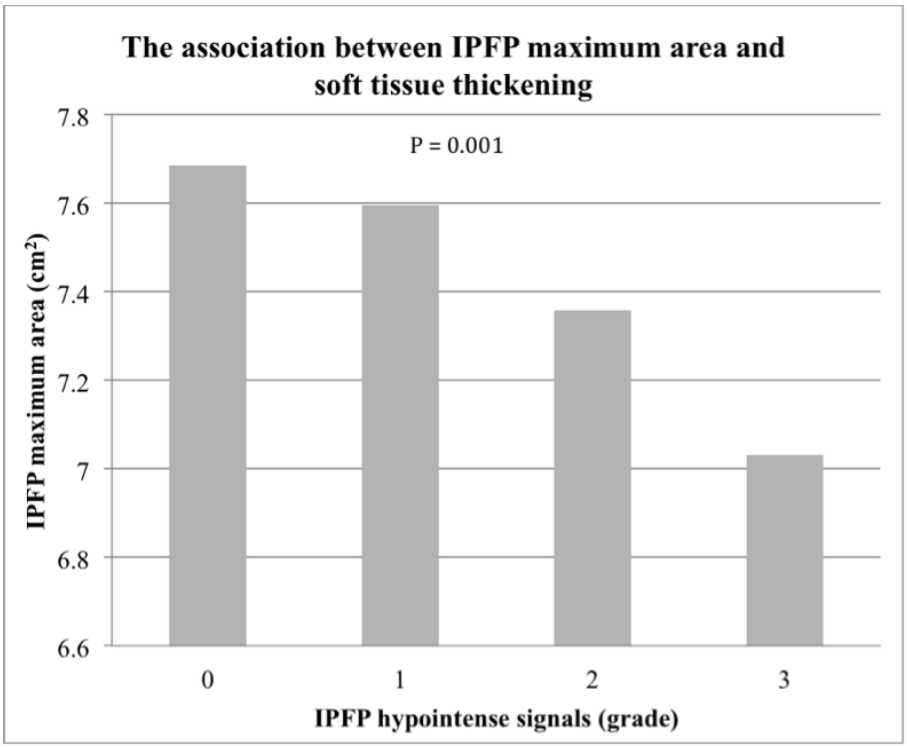

Figure B.1 Association between IPFP maximum area and hypointense signals. IPFP: infrapatellar fat pad

Appendix C Appendices for Chapter 6

Table C.1 Cross-sectional associations between IPFP signal intensity alteration and ROA

	Univariable β (95% CI)	Multivariable* β (95% CI)
sDev (IPFP)	**0.58 (0.29, 0.88)**	**0.61 (0.31, 0.91)**
UQ (H)	**0.87 (0.17, 1.56)**	**0.96 (0.27, 1.65)**
Percentage (H)	**0.77 (0.30, 1.25)**	**0.65 (0.19, 1.11)**
Clustering factor (H)	**0.86 (0.13, 1.59)**	**1.27 (0.36, 2.18)**

Dependent variable: ROA; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, sex and BMI.

IPFP: infrapatellar fat pad; ROA: radiographic osteoarthritis; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

Table C.2 Cross-sectional associations between IPFP signal intensity alteration and cartilage defects

	Univariable OR (95% CI)	Multivariable* OR (95% CI)
Baseline tibiofemoral cartilage defects		
sDev (IPFP)	**1.19 (1.06, 1.34)**	**1.22 (1.08, 1.39)**
UQ (H)	**1.35 (1.03, 1.77)**	**1.40 (1.06, 1.85)**
Percentage (H)	**1.44 (1.19, 1.74)**	**1.42 (1.18, 1.72)**
Clustering factor (H)	**2.11 (1.45, 3.08)**	**2.18 (1.49, 3.20)**
Baseline patellar cartilage defects		
sDev (IPFP)	0.00 (-0.02, 0.02)	0.01 (-0.01, 0.03)
UQ (H)	0.00 (-0.04, 0.04)	0.01 (-0.03, 0.05)
Percentage (H)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)
Clustering factor (H)	0.00 (-0.06, 0.06)	0.01 (-0.05, 0.07)

Dependent variable: cartilage defects; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, sex, and BMI.

IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

Table C.3 Cross-sectional associations between IPFP signal intensity alteration and BMLs

	Univariable OR (95% CI)	Multivariable* OR (95% CI)
Baseline tibiofemoral BMLs		
sDev (IPFP)	**1.24 (1.06, 1.42)**	**1.26 (1.09, 1.45)**
UQ (H)	**1.41 (1.05, 1.90)**	**1.43 (1.05, 1.93)**
Percentage (H)	**1.43 (1.17, 1.74)**	**1.42 (1.16, 1.73)**
Clustering factor (H)	**2.34 (1.55, 3.54)**	**2.36 (1.56, 3.57)**
Baseline patellar BMLs		
sDev (IPFP)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.03)
UQ (H)	0.01 (-0.04, 0.05)	0.01 (-0.03, 0.05)
Percentage (H)	0.01 (-0.03, 0.04)	0.01 (-0.03, 0.04)
Clustering factor (H)	0.01 (-0.06, 0.08)	0.02 (-0.05, 0.08)

Dependent variable: BMLs; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, sex, and BMI.

BMLs: bone marrow lesions; IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity

Table C.4 Cross-sectional associations between IPFP signal intensity alteration and cartilage volume

	Univariable β (95% CI)	Multivariable* β (95% CI)
Baseline tibial cartilage volume		
sDev (IPFP)	**0.08 (0.02, 0.14)**	-0.01 (-0.05, 0.04)
UQ (H)	**0.17 (0.04, 0.31)**	0.02 (-0.08, 0.13)
Percentage (H)	**-0.10 (-0.19, -0.01)**	**-0.09 (-0.15, -0.02)**
Clustering factor (H)	0.01 (-0.14, 0.15)	**-0.15 (-0.28, -0.01)**
Baseline patellar cartilage volume		
sDev (IPFP)	0.01 (-0.03, 0.04)	**-0.05 (-0.08, -0.02)**
UQ (H)	-0.01 (-0.09, 0.07)	**-0.09 (-0.15, -0.04)**
Percentage (H)	0.01 (-0.06, 0.07)	0.01 (-0.03, 0.06)
Clustering factor (H)	0.03 (-0.09, 0.16)	-0.05 (-0.14, 0.04)

Dependent variable: cartilage volume; Independent variable: IPFP signal intensity alteration.

*Adjusted for age, gender, BMI and bone area.

IPFP: infrapatellar fat pad; sDev (IPFP): standard deviation of IPFP signal intensity values; UQ(H): upper quartile value of high signal intensity region; Percentage (H): ratio of volume of high signal intensity region/whole IPFP volume; Clustering factor(H): clustering factor of high signal intensity.

Appendix D Published Manuscripts

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Arthritis Research & Therapy

RESEARCH ARTICLE Open Access

Hypointense signals in the infrapatellar fat pad assessed by magnetic resonance imaging are associated with knee symptoms and structure in older adults: a cohort study

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Abstract

Background: There are few clinical and epidemiological studies reporting the association between abnormal changes within the IPFP and knee osteoarthritic changes. This study aims to describe the associations between hypointense signals in the infrapatellar fat pad (IPFP) and knee structural change and symptoms in older adults.

Methods: Participants ($n = 874$) were selected randomly from local community and followed up 2.7 years later (range 2.6–3.3 years). T1- or T2-weighted fat-suppressed magnetic resonance imaging (MRI) was assessed for IPFP hypointense signal, cartilage volume, cartilage defects, and bone marrow lesions (BMLs). Knee pain was assessed by self-administered Western Ontario and McMaster Osteoarthritis Index (WOMAC) questionnaire. Radiographic osteoarthritis was assessed using the OARSI atlas.

Results: Cross-sectionally, hypointense signals in the IPFP were significantly associated with a higher risk of knee cartilage defects at all sites, tibiofemoral BMLs and knee pain in multivariable analyses. Longitudinally, baseline signal abnormalities were significantly and positively associated with increases in knee cartilage defects (OR: 2.27, 95 % CI: 1.61–3.21), BMLs (OR: 1.91, 95 % CI: 1.39–2.62), and knee pain (OR: 1.36, 95 % CI: 1.05–1.76) in multivariable analyses. The associations with cartilage defects remained significant after adjustment for BMLs, but the associations with BMLs and knee pain decreased in magnitude or became non-significant after further adjustment for cartilage defects.

Conclusions: Hypointense signals in the IPFP were associated primarily with increased knee cartilage defects and also with BMLs and knee symptoms in cross-sectional and longitudinal analyses, suggesting the abnormality represented by this signal has a potentially important role in osteoarthritis progression.

Keywords: Infrapatellar fat pad, Osteoarthritis, Signal intensity, Cartilage defects, Bone marrow lesions, Pain

Background

Osteoarthritis (OA) is the most prevalent chronic joint disorder, characterized by pain and progressive deterioration of joint structures, and is strongly associated with risk factors such as age, female sex and obesity [1]. The most commonly affected joint is the knee, and the whole knee joint structures including articular cartilage, subchondral bone, synovium, ligaments, meniscus and periarticular fat pad can be affected in the course of knee OA [2]. Imaging biomarkers, especially from magnetic resonance imaging (MRI), have been used in OA research for over a decade [3]. Because of its advantage in direct visualization of morphology and integrity of the whole joint, MRI has been considered as a sensitive and accurate tool to assess cartilage loss, subchondral bone abnormalities, synovitis, and ligament and meniscal lesions [4–6]. Quantitative or semiquantitative scoring

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systems have been developed for evaluating these structural changes in OA [4–6].

The infrapatellar fat pad (IPFP), the local fat around the knee joint, may play an important role in the initiation and progression of knee OA [7, 8]. Biomechanically, it can promote efficient lubrication, reduce impact loading and absorb forces generated through the knee joint, which may be protective against OA [9]. Biochemically, it can produce various pro-inflammatory cytokines and adipokines, which may be deleterious to the knee joint [10–13]. Pathological examination of IPFP obtained from patients with end-stage OA found that vascular neoformations, fibrosis, and chronic inflammation were present in these specimens [14]. Dragoo et al. have suggested that sagittal MRI can be used to assess abnormal IPFP quality, including fibrosis, inflammation, oedema and mass-like lesions [15].

So far, there are few clinical and epidemiological studies reporting the association between abnormal changes within the IPFP and knee osteoarthritic changes. Higher signal intensity change around the IPFP assessed by T2-weighted MRI has been considered as a surrogate for peripatellar synovitis [16, 17]. Our previous study reported that high signal intensity alteration within the IPFP was associated with knee symptoms and structural changes in older adults [18]. While hyperintense signals within the IPFP on T2-weighted MRI can indicate inflammation, acute haemorrhage and/or oedema, hypointense signals within the IPFP on T2-weighted MRI may indicate fibrosis [15]. So far, there are no studies reporting whether hypointense signals within the IPFP are associated with symptoms and structures in knee OA. The aim of this study was to describe whether hypointense signals within the IPFP measured by T2-weighted MRI are associated with symptoms or joint structural abnormalities cross-sectionally and longitudinally in older adults.

Methods

Subjects

This study was conducted as part of the Tasmanian Older Adult Cohort (TASOAC) study, an ongoing prospective, population-based study that was aimed at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA. Men and women aged 50–80 years in 2002 were selected from the electoral roll in southern Tasmania (population, 229,000) using sex-stratified simple random sampling without replacement (response rate, 57 %). Baseline measures were conducted from April 2002 to September 2004, and the follow-up was conducted from September 2004 to February 2007 (mean 2.7 years, range 2.6–3.3 years). Institutionalized persons and subjects with contraindications to MRI and diagnosed rheumatoid arthritis were excluded. This study consisted of 874 participants who had knee MRI scans at baseline.

Anthropometrics

Height was measured to the nearest 0.1 cm (with shoes, socks, and headgear removed) using a stadiometer. Weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) by using a single pair of electronic scales (Delta Model 707, Seca, Hamburg, Germany) that were calibrated using a known weight at the beginning of each clinic. Body mass index [BMI, weight (kg)/height (m^2)] was also calculated.

WOMAC pain assessment

The assessment of knee pain (when walking on flat surface, when going up/down stairs, at night while in bed, when sitting/lying and when standing) was self-administered, using the Western Ontario and McMaster Osteoarthritis Index (WOMAC) with a 10-point scale from 0 (no pain) to 9 (most severe) [19]. A total score for knee pain (0 to 45) was determined by each component score, and the presence of knee pain was defined as a total score or a subscale score of ≥ 1. An increase in knee pain was defined as a change in the score of ≥ 1.

Knee radiographic assessment

All subjects performed a standing anteroposterior semi-flexed view of the right knee with 15 degrees of fixed knee flexion, and radiographs were individually assessed for joint space narrowing (JSN) and osteophytes on a scale of 0–3 (0 = normal and 3 = most severe) by using the Osteoarthritis Research Society International (OARSI) atlas developed by Altman et al. [20]. The osteophytes and JSN scores were summed as the knee total radiographic OA (ROA) score, of which 1 or greater was used to define the presence of knee ROA, as previously described [21].

Magnetic resonance imaging assessment

MRI scans of the right knees were performed at baseline and follow-up. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Picker, Cleveland, OH, USA) with the use of a commercial transmit-receive extremity coil. The following image sequences were used: (1) a T1-weighted fat saturation three-dimensional gradient recall acquisition in the steady state; flip angle 30 degrees; repetition time 31 ms; echo time 6.71 mc; field of view 16 cm; 60 partitions; 512 × 512 matrix; acquisition time 11 min 56 sec; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.31 (512 × 512 pixels); (2) a T2-weighted fat saturation two-dimensional fast spin echo, flip angle 90°, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 256 × 256-pixel matrix; sagittal images were obtained at a slice thickness of 4 mm with a interslice gap of 1.0 mm.

Hypointense signals within the IPFP were scored by counting imaging slices with this abnormality: grade 0 = none; grade 1 = 1–2 slices, grade 2 = 3–5 slices, grade 3 = ≥6 slices. This measurement was conducted by two experienced orthopaedists (HW and ZZ) trained by an experienced radiologist (HA), and determined using T2-weighted MR images (Fig. 1). Intraobserver and interobsever reliabilities were assessed in 100 subjects with an intraclass correlation coefficient (ICC) of 0.94 and an interobserver correlation coefficient of 0.88.

Knee cartilage volume was assessed on T1-weighted MR images with image processing on an independent workstation, as previously described [22–24]. The total cartilage volume was divided into patellar, medial and lateral tibial cartilage volume by manually drawing disarticulation contours around the cartilage boundaries, section by section, which were then resampled for the final three-dimensional rendering [22, 23]. The coefficients of variation (CVs) for this method in our hands were 2.1–2.6 % [22, 23]. Changes in cartilage volume were calculated as: change per annum = (follow-up volume − baseline volume)/time between two scans in years.

Cartilage defects (0–4 scale) were determined at the medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites as previously described [25, 26] as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface; grade 2 = irregularities on the surface or bottom and loss of thickness < 50 %; grade 3 = deep ulceration with loss of thickness > 50 %; grade 4 = full-thickness chondral wear with exposure of subchondral bone. The presence of cartilage defects was defined as a cartilage defect score of ≥ 2 at any site. Intraobserver reliabilities were 0.89–0.94 and interobserver reliabilities were 0.85–0.93 [25]. An increase in cartilage defects was defined as a change in cartilage defects of ≥ 1.

Subchondral bone marrow lesions (BMLs) were defined as discrete areas of increased signal adjacent to the subcortical bone at the medial and lateral tibia and femur on T2-weighted MR images using a semi-quantitative (0–3) scoring system. The intraobserver reliability ranged between 0.89–1.00, as previously described [27]. An increase in BMLs was defined as a change in BMLs of ≥ 1.

Tibial plateau bone area was determined by manually measuring on axial T1-weighted MR images, as previously described [21].

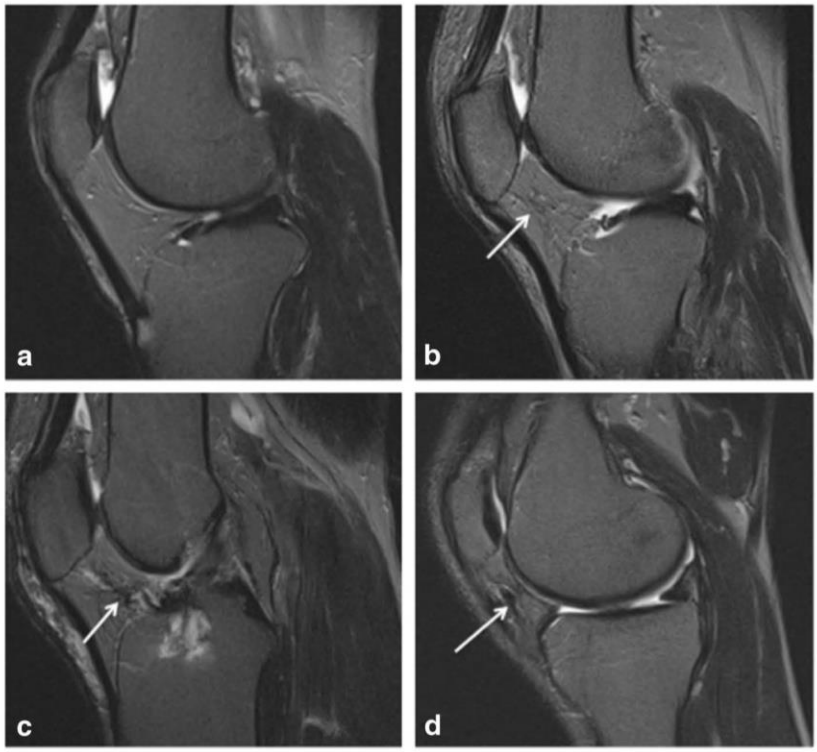

Fig. 1 Hypointense signals on sagittal T2-weighted images with fat saturation. **a** Normal IPFP; **b** grade 1 hypointense signals of IPFP (*arrow*); **c** grade 2 hypointense signals of IPFP (*arrow*); **d** grade 3 hypointense signals of IPFP (*arrow*)

Data analysis

Student t or χ^2 tests were used to compare means or proportions, respectively. Multivariable linear regression analyses were used to examine the associations between IPFP hypointense signals (independent variable) and knee cartilage volume or change in cartilage volume (dependent variables) after adjustment for age, sex, BMI, ROA, tibial bone area, and/or baseline cartilage volume (for change in cartilage volume) with further adjustment for cartilage defects or BMLs. Multivariable binary logistic regression analyses were used to examine the associations between IPFP hypointense signals (independent variable) and presences of knee joint space narrowing, osteophytes, as well as baseline or increases in knee cartilage defects, BMLs and WOMAC measures (dependent variables), after adjustment for covariates.

A p value < 0.05 (two-tailed) or a 95 % confidence interval (CI) not including the null point (for linear regression) or 1 (for logistic regression) was considered as statistical significance. All statistical analyses were performed on IBM SPSS version 20.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

A total of 874 subjects between 50 and 80 years of age (mean, 62.1 years) took part in the present study. There were no significant differences in demographic factors (age, sex, and BMI) between these participants and those excluded (n = 226) (data not shown). Over 2.6 years, 104 subjects were lost to follow-up study due to: 25 deceased, 18 moved to other states or overseas, 12 had joint replacement, 24 physically unable, and 25 no reason specified. The remaining 770 subjects completed the follow-up study and the first 357 had the second MRI scans but not the others as the MRI machine in the hospital was decommissioned. There were no significant differences between these subjects and those without follow-up MRI, as previously described [28].

Table 1 describes characteristics of the study population. There was no significant difference in age, patellar cartilage volume and knee pain between subjects with and without IPFP hypointense signals; but the group with IPFP hypointense signals had a greater proportion of men, and higher prevalence of JSN, osteophytes, BMLs, cartilage defects, as well as higher BMI. Additionally, these subjects had greater tibial cartilage volume and tibial bone area.

IPFP hypointense signals were significantly and positively associated with baseline (data not shown) and increases in (Fig. 2) cartilage defects at all compartments in unadjusted analyses. They were significantly and positively associated with all cartilage defects after adjustment for age, sex, BMI, and radiographic OA cross-sectionally and longitudinally (Table 2). These associations remained significant after further adjustment for BMLs, except that the longitudinal association at the patellar site decreased in magnitude and became of borderline significance (Table 2).

Cross-sectionally, IPFP hypointense signals were not significantly associated with medial and lateral tibial cartilage volume, but significantly and negatively associated with patellar cartilage volume after adjustment for age, sex, BMI, radiographic OA and tibial bone area (Additional file 1: Table S1). This significant association disappeared after further adjustment for patellar cartilage defects but remained unchanged after further adjustment for patellar BMLs (Additional file 1: Table S1). Longitudinally, IPFP hypointense signals were negatively and significantly associated with change in lateral tibial cartilage volume, but not with changes in medial tibial and patellar cartilage volume, after adjustment for age, sex, BMI, radiographic OA, tibial bone area and baseline cartilage volume, and this association remained after further adjustment for cartilage defects or BMLs (Additional file 1: Table S1).

In cross-sectional analyses, IPFP hypointense signals were significantly and positively associated with any BMLs and tibiofemoral BMLs after adjustment for age, sex, BMI and radiographic OA. The associations decreased in magnitude and became non-significant after further adjustment for cartilage defects (Table 3). Longitudinally, IPFP hypointense signals were significantly and positively associated with increases in BMLs at all sites before (Fig. 2) and after adjustment for age, sex, BMI and radiographic OA (Table 3). After further adjustment for cartilage defects, the associations decreased in magnitude and became non-significant at lateral tibiofemoral and patellar sites (Table 3).

IPFP hypointense signals were significantly and positively associated with total knee pain, pain when going up/down stairs, at night while in bed, and when sitting/lying after adjustment for age, sex, BMI, and radiographic OA in cross-sectional analyses, but these significant associations disappeared after further adjustment for cartilage defects or BMLs except for pain when at night while in bed (Table 4). Longitudinally, IPFP hypointense signals were significantly associated with an increase in total knee pain, pain when walking on a flat surface, pain when going up/down stairs and when standing (Table 4), but these became non-significant after further adjusting for cartilage defects. The associations between IPFP hypointense signals and an increase in knee pain decreased in magnitude but became non-significant for all knee pain subscales (except pain when going up/down stairs) after further adjustment for BMLs (Table 4).

In cross-sectional analyses, IPFP hypointense signals were significantly and positively associated with ROA (OR: 2.91, $p < 0.001$), tibiofemoral joint space narrowing

Table 1 Baseline characteristics of participants split by presence of IPFP hypointense signal

	IPFP hypointense signal No (*N* = 305)	IPFP hypointense signal YES (*N* = 569)	*p* value
Age (year)	61.6 (6.9)	62.4 (7.5)	0.090
Female sex (%)	**65.9**	**41.7**	**<0.001**
Body mass index (kg/m^2)	**27.0 (4.5)**	**28.1 (4.7)**	**<0.001**
Medial tibial cartilage volume (ml)	**2.2 (0.6)**	**2.4 (0.6)**	**<0.001**
Lateral tibial cartilage volume (ml)	**2.6 (0.7)**	**2.8 (0.7)**	**<0.001**
Patella cartilage volume (ml)	3.2 (0.9)	3.2 (1.0)	0.235
Medial tibial bone area (cm^2)	**19.9 (2.9)**	**21.4 (3.0)**	**<0.001**
Lateral tibial bone area (cm^2)	**11.4 (1.9)**	**12.5 (2.2)**	**<0.001**
Medial joint space narrowing (%)	**32.9**	**63.3**	**<0.001**
Lateral joint space narrowing (%)	**15.2**	**28.2**	**<0.001**
MTF osteophytes (%)	**1.8**	**10.0**	**<0.001**
LTF osteophytes (%)	**0.7**	**5.5**	**0.001**
BML present (%)	**29.2**	**40.1**	**0.001**
MTF cartilage defects (%)	**10.2**	**30.8**	**<0.001**
LTF cartilage defects (%)	**11.3**	**26.7**	**<0.001**
Patellar cartilage defects (%)	**29.4**	**44.3**	**<0.001**
Knee pain (%)	46.2	51.4	0.144

Two-tailed *t* tests were used for differences between means, and χ2 tests were used for proportions (percentages). Significant differences are shown in bold. Mean (SD) except for percentages
IPFP infrapatellar fat pat, *MTF* medial tibiofemoral, *LTF* lateral tibiofemoral, *BML* bone marrow lesions

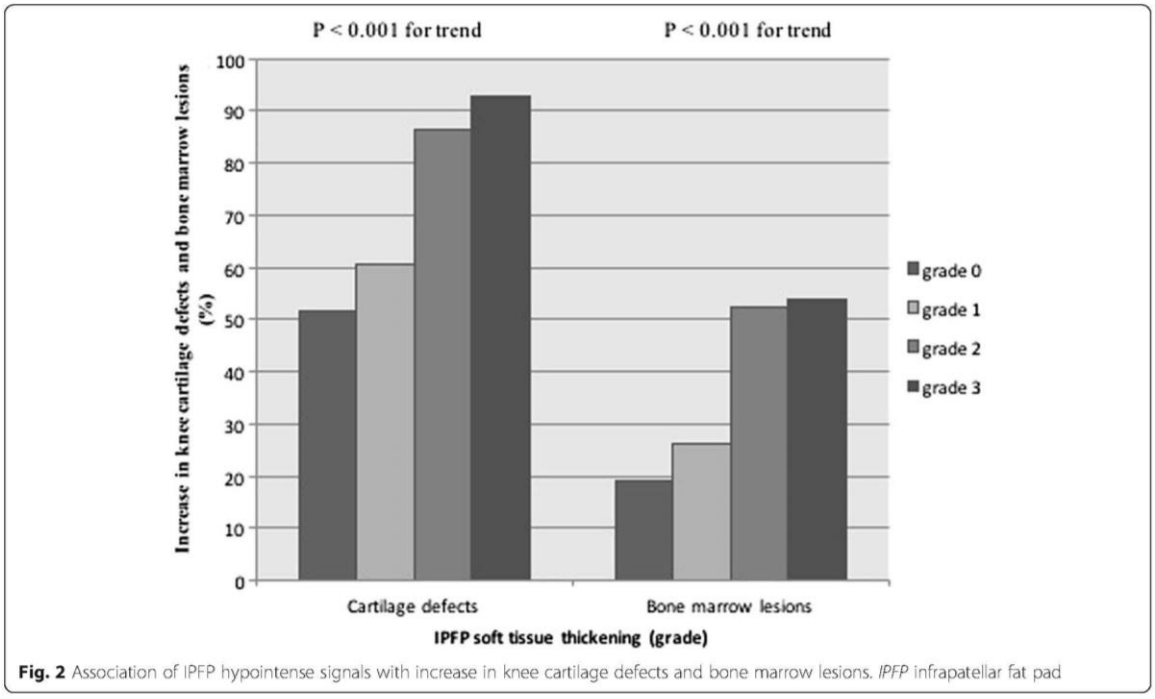

Fig. 2 Association of IPFP hypointense signals with increase in knee cartilage defects and bone marrow lesions. *IPFP* infrapatellar fat pad

Table 2 Associations of IPFP hypointense signals with baseline knee cartilage defects and increases in knee cartilage defects over 2.6 years

	Multivariable[a] OR (95 % CI)	Multivariable[b] OR (95 % CI)
Baseline cartilage defects		
Any cartilage defects	2.38 (1.87, 3.03)	2.24 (1.74, 2.87)
Medial tibiofemoral	2.93 (2.24, 3.83)	2.70 (2.05, 3.54)
Lateral tibiofemoral	2.62 (2.02, 3.42)	2.57 (1.95, 3.38)
Patellar	1.84 (1.47, 2.31)	1.93 (1.50, 2.47)
Increase in knee cartilage defects		
Any cartilage defects	2.27 (1.61, 3.21)	2.08 (1.46, 2.97)
Medial tibiofemoral	1.62 (1.21, 2.19)	1.55 (1.15, 2.09)
Lateral tibiofemoral	1.46 (1.09, 1.96)	1.39 (1.03, 1.88)
Patellar	1.38 (1.00, 1.90)	1.36 (0.99, 1.88)

Dependent variables: baseline cartilage defects or increases in knee cartilage defects (yes v no); independent variables: IPFP hypointense signals (per grade)
IPFP infrapatellar fat pat
[a]Adjusted for age, sex, BMI, and radiographic osteoarthritis
[b]Further adjustment for bone marrow lesions

(OR: 2.72 and 1.59, respectively, for medial and lateral compartments; both $p < 0.01$), and tibiofemoral osteophytes (OR: 4.25 and 3.34, respectively, for medial and lateral compartments; both $p < 0.001$), after adjustment for age, sex and BMI.

Higher grade of IPFP hypointense signals was significantly associated with smaller IPFP maximal area after adjustment for age, gender, BMI, and total tibial bone area (Additional file 2: Figure S1). The associations of IPFP hypointense signals with the above outcome measures remained unchanged after further adjustment for IPFP maximal area (data not shown).

Table 3 Associations between IPFP hypointense signals and baseline bone marrow lesions and increases in bone marrow lesions over 2.6 years

	Multivariable[a] OR (95 % CI)	Multivariable[b] OR (95 % CI)
Baseline bone marrow lesions		
Any bone marrow lesions	1.64 (1.32, 2.03)	1.11 (0.87, 1.42)
Medial tibiofemoral	1.77 (1.39, 2.25)	1.24 (0.95, 1.63)
Lateral tibiofemoral	1.40 (1.08, 1.80)	1.03 (0.78, 1.36)
Patellar	1.21 (0.93, 1.57)	0.83 (0.61, 1.13)
Increases in bone marrow lesions		
Any bone marrow lesions	1.91 (1.39, 2.62)	1.45 (1.02, 2.04)
Medial tibiofemoral	2.11 (1.46, 3.07)	1.59 (1.06, 2.39)
Lateral tibiofemoral	1.66 (1.17, 2.35)	1.36 (0.93, 1.97)
Patellar	1.50 (1.04, 2.15)	1.34 (0.92, 1.95)

Dependent variables: baseline bone marrow lesions or increases in bone marrow lesions (yes v no); independent variables: IPFP hypointense signals (per grade)
IPFP infrapatellar fat pat
[a]Adjusted for age, sex, BMI, and radiographic osteoarthritis
[b]Further adjustment for cartilage defects

Discussion

This study is the first study to investigate the association of IPFP hypointense signals with knee structural and symptom changes in older adults. We found that IPFP hypointense signals were cross-sectionally associated with increased knee symptoms, cartilage defects, BMLs, and radiographic OA, and with reduced patellar cartilage volume and IPFP maximal area. Longitudinally, these signal intensity changes predicted increases in cartilage defects at all sites and BMLs, loss of lateral tibial cartilage volume and increases in knee symptoms. The associations with knee cartilage defects remained significant after adjustment for BMLs, but the associations with BMLs and knee pain were weakened after adjustment for cartilage defects. This suggests that IPFP hypointense signals are associated with increased knee cartilage defects primarily and with knee BMLs and pain secondarily in older adults.

IPFP, an intracapsular but extrasynovial structure [29], is situated in the knee under the patella, between the patellar tendon, femoral condyle and tibial plateau [7], and is structurally similar to subcutaneous adipose tissue [30]. As a deformable soft tissue within the anterior compartment of the knee joint, it can adapt to change contours of the articular surface and is able to distribute synovial fluid within the joint cavity to reduce the articular surface cushion, besides supporting the feasibility of intra-articular ligaments [9]. Composed of a fibrous scaffold with adipose tissue and synovial recesses within it, the IPFP can secrete cytokines, adipokines, and lipid mediators [10–13, 31]. Therefore, the IPFP may play a biphasic role in the pathologic progression of knee abnormalities.

MRI has been used to assess signal alterations within or around the IPFP, and the high signal intensity alteration was mainly considered as synovial inflammation or Hoffa synovitis [16, 32]. In addition, hypointense signals closing to the synovium within or around the IPFP was regarding as chronic synovitis [33, 34]. Hoffa synovitis is recognised as a key imaging biomarker for knee OA [6], and can predict the progression of knee OA [32]. Our previous study also suggested that high signal intensity alteration within the IPFP was associated with knee structural and symptomatic changes in older adults [18]. As IPFP signal intensity alteration may reflect different pathological changes, more studies are required to assess the roles of IPFP signal intensity changes in knee OA.

So far, there have been no studies reporting the clinical significance of IPFP hypointense signals observed on T2-weighted fat-saturated MRI images. Low signal intensity changes within the IPFP may represent fibrosis

Table 4 Association of IPFP hypointense signals with WOMAC measures and increases in WOMAC measures over 2.6 years

	Multivariable[a] OR (95 % CI)	Multivariable[b] OR (95 % CI)	Multivariable[c] OR (95 % CI)
Baseline WOMAC measures			
Total knee pain	1.27 (1.03, 1.56)	1.07 (0.85, 1.35)	1.15 (0.93, 1.43)
Pain on flat surface	1.22 (0.97, 1.55)	1.02 (0.79, 1.33)	1.13 (0.88, 1.44)
Pain on stairs	1.26 (1.02, 1.55)	1.05 (0.84, 1.32)	1.14 (0.92, 1.42)
Pain in bed	1.47 (1.16, 1.85)	1.42 (1.10, 1.82)	1.42 (1.12, 1.79)
Pain when sitting	1.29 (1.02, 1.64)	1.25 (0.96, 1.62)	1.24 (0.97, 1.58)
Pain when standing	1.22 (0.96, 1.55)	1.10 (0.85, 1.43)	1.12 (0.87, 1.43)
Increases in WOMAC measures			
Total knee pain	1.36 (1.05, 1.76)	1.32 (0.99, 1.74)	1.30 (1.00, 1.69)
Pain on flat surface	1.52 (1.08, 2.14)	1.22 (0.83, 1.79)	1.33 (0.94, 1.89)
Pain on stairs	1.51 (1.14, 2.01)	1.34 (0.98, 1.82)	1.42 (1.06, 1.89)
Pain in bed	1.18 (0.85, 1.63)	1.04 (0.73, 1.49)	1.14 (0.82, 1.58)
Pain when sitting	1.25 (0.88, 1.78)	1.14 (0.77, 1.67)	1.20 (0.84, 1.71)
Pain when standing	1.44 (1.02, 2.03)	1.22 (0.84, 1.78)	1.38 (0.98, 1.97)

Dependent variables: baseline WOMAC measures or increases in WOMAC measures (yes vs. no); independent variables: IPFP hypointense signals (per grade)
IPFP infrapatellar fat pat, *WOMAC* Western Ontario and McMasters osteoarthritis index
[a]Adjusted for age, sex, BMI, and radiographic osteoarthritis
[b]Further adjustment for cartilage defects
[c]Further adjustment for bone marrow lesions but not for cartilage defects

or postoperative scarring [29, 35], chronic inflammation progressing from acute inflammation of the synovium due to microtrauma of this tissue [36], or synovial thickening or fibrosis [33, 37]. A study compared low signal intensity changes in plantar fat pad on MRI with histological changes and reported that these signal changes corresponded to fibrosis [38]. This fibrosis can be induced by chronic inflammation in the synovium [36], or periarticular surgeries or trauma around knees [29]. Although the roles of synovitis, surgical history and trauma in knee OA have been identified [39, 40], there has been no evidence showing that low signal intensity within IPFP assessed by MRI is associated with symptoms and knee structural changes in the knee.

In this study, we found that in older adults, IPFP hypointense signals were consistently associated with knee cartilage defects cross-sectionally and longitudinally, independent of factors such as BMLs. They were also associated with BMLs and reduced cartilage volume cross-sectionally and longitudinally, but these associations were largely dependent of cartilage defects. Further, there was significant association between IPFP hypointense signals and knee pain, but again, these associations were largely dependent of cartilage defects rather than BMLs. These suggest that IPFP hypointense signals may induce knee structural changes and symptoms starting from cartilage defects.

The mechanisms underlying the associations between IPFP low signal intensity and knee OA measures are largely unknown. These hypointense signals within the synovial membrane have been considered as synovial fibrosis and were corresponding to chronic synovitis [33, 34]. This chronic synovitis can contribute to cartilage degradations and knee pain [41]. Other pathological changes such as adipocyte necrosis or adipose fibrosis may be observed as hypointense signals within the IPFP in MR imagines. Fibrosis is an abnormal tissue healing process that occurs sequentially from an inflammatory response to surgery or injury of the knee and severe fibrosis was found in 33 % of IPFP biopsy specimens resected from patients with end-stage knee OA [14]. Fibrosis was also found in monoiodoacetate-induced OA models and was associated with knee pain [36]. In addition, a study focusing on the effect of strenuous running on IPFP histological changes in a rat OA model reported that fibrosis within IPFP was associated with excess physical activities and related to knee pain [42]. Fibrosis within IPFP may increase cartilage contact pressures and decrease the ability of absorbing the shock through the knee, and thus induce the degradation of neighbouring knee structures including cartilage and subchondral bone. Furthermore, our current study found that this abnormal signal was negatively associated with IPFP maximum area, suggesting it may decrease this absorbing ability through reducing the IPFP size.

The main strength of this cohort study lies in a large sample size with the comprehensive MRI structural measurements. There are several potential limitations. First, the response rate at baseline was 57 %, possibly due to the extensive protocol, which did leave the possibility open for

selection bias. However, there were no significant differences in age, gender and BMI between those who responded and those did not. We also had high rates of retention (82 %) to offset this. Second, we did not have radiographic OA measurements at the follow-up because X-ray is insensitive for change over this short period, so we are unable to determine the association between IPFP quality and change in radiographic OA. Third, measurement error may influence results. However, all measures were highly reproducible suggesting this is unlikely. Lastly, histological examinations were not able to be performed in this community-based study, so the pathological changes associated with IPFP low signal intensity are unknown.

Conclusions

In conclusion, hypointense signals in the IPFP were associated primarily with increased knee cartilage defects and also with BMLs and knee symptoms in cross-sectional and longitudinal analyses, suggesting the abnormality represented by this signal has a potentially important role in osteoarthritis progression.

Additional files

Additional file 1: Table S1. Associations of IPFP hypointense signals with baseline knee cartilage volume and change in knee cartilage volume over 2.6 years. (DOC 31 kb)

Additional file 2: Figure S1. Association between IPFP maximum area and hypointense signals. *IPFP* infrapatellar fat pad. (PNG 59 kb)

Abbreviations

BMI: body mass index; BMLs: bone marrow lesions; CI: confidence interval; CVs: coefficients of variation; ICC: intraclass correlation coefficient; IPFP: infrapatellar fat pad; JSN: joint space narrowing; MRI: magnetic resonance imaging; OA: osteoarthritis; OARSI: Osteoarthritis Research Society International; ROA: radiographic osteoarthritis; TASOAC: Tasmanian Older Adult Cohort; WOMAC: Western Ontario and McMaster Osteoarthritis

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Availability of data and materials

Not applicable.

Authors' contributions

CD had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. CD carried out the study design, participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis. WH participated in the acquisition, analysis and interpretation of data, manuscript preparation, and statistical analysis. ZZ participated in the acquisition of data, manuscript preparation, and statistical analysis. DA participated in the analysis and interpretation of data, manuscript preparation, and statistical analysis. AH participated in the acquisition, analysis and interpretation of data, and manuscript preparation. XW participated in the acquisition of data, manuscript preparation, and statistical analysis. BA participated in the acquisition, analysis and interpretation of data, and manuscript preparation. FC participated in the study design, acquisition of data, manuscript preparation, and statistical analysis. GJ participated in the study design, acquisition, analysis and interpretation of data, and manuscript preparation. All authors read and approved the final manuscript.

Authors' information

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

We certify that the contents of this article have not been submitted or published elsewhere and written permission has been obtained from all persons named in the acknowledgment. We have not published or submitted any related articles from the same study. We also certify that we meet published criteria for authorship.

Ethics approval and consent to participate

TASOAC study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and written informed consents were obtained from all participants.

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